

PLANT PHYSIOLOGY

1654/B

EDITORS

CHARLES A. SHULL

CHARLES B. LIPMAN

BURTON E. LIVINGSTON

CARLETON R. BAIL

FRANCIS E. LLOYD

VOLUME II

1927

WITH FIVE PLATES AND EIGHTY-SIX FIGURES

Reprinted by arrangement with the original publishers

JOHNSON REPRINT CORPORATION

NEW YORK, NEW YORK

First reprinting, 1958, Johnson Reprint Corporation

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ERRATA, VOLUME II

- Page 132, line 5, for investigation read investigations.
 Page 164, line 14, for Schaffer read Shaffer.
 Page 179, line 1, for is read in.
 Page 205, line 8, for (10) read (12).
 Page 206, line 14, for (11) read (13).
 Page 205, line 35, for (6) read (6, 7).
 Page 207, line 18, for (9) read (10, 11).
 Page 208, line 14, for (8) read (9).
 Page 209, line 19, for (7) read (8).
 Page 221, line 4, for wil read will.
 Page 224, line 7, for soils read sols.

PLANT PHYSIOLOGY

JANUARY, 1927

NEGATIVE RESULTS ON PHYSIOLOGICAL BALANCE IN SOIL CULTURES*

R. P. HIBBARD

(WITH ELEVEN FIGURES)

Introduction

The use in the field of the triangular system of determining salt ratios was first attempted by the writer in the growing season of 1918. In the fall of that year, SCHREINER and SKINNER (10) reported on some of their field work. It has been shown previously by the author (3) that there is some evidence of a physiological balance in the soil solution extracted from the soil and set up in culture jars in the greenhouse. The study reported here was expected to show whether or not a physiological balance exists in the soil solution *in situ*. It is perfectly evident that such a study as this is considerably more complicated than one in water cultures. The medium of growth, a fertile soil, is indeed very complex, physically, chemically, and biologically. It must also be considered as in a dynamic state, in a condition of change. In spite of the complexities which make correct interpretations difficult, and because there are still possibilities for improvement in the generally accepted plan of applying soil fertilizers, SCHREINER and SKINNER (10), LIPMAN and LINHART (7), efforts should be made and are being made to apply the triangular system to field studies. It is quite evident that for these reasons, if not for the fact that our annual expenditure for fertilizer practice is well over a hundred million dollars a year, any and every fertilizer practice should be actively investigated.

Such studies involve investigations of the salt requirements of agricultural plants, a study of the mechanism of absorption, and of the uses, functions and modifications of the various ions when finally within the plant.

* Published with the permission of the Director of the Experiment Station.

Greenhouse methods

The method of conducting this experiment is based on previous but unpublished studies of wheat, corn, and tomatoes grown in different media, soil, quartz sand, and distilled water (4).

The culture pots were 23 cm. high and 20.5 cm. in diameter, sufficiently large to hold enough soil (6 kilograms) to allow for the growth of wheat to maturity. In using pots of this size it is necessary to construct special watering devices, incorrectly designated by some authors as potometers. The apparatus has been frequently used in this laboratory for many years and is described by YUNCKER (16).

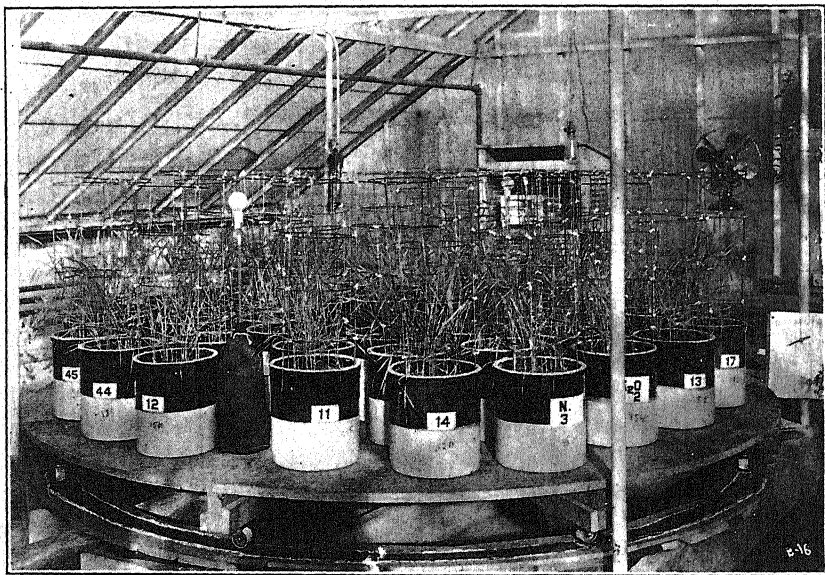


FIG. 1. Plant cultures in early stages of growth on rotating table. Temperature cage, evaporimeters, and humidity apparatus are shown.

For aerating the culture, a piece of glass tubing was run into the soil down to the gravel layer at the bottom of the crock. The old air was forced out and new introduced each time water was added through the watering device. Since the surface was not sealed with wax, evaporation from the soil was lessened by a top layer of fine gravelly loam. As a record of water lost from the plants was desired, the small amount lost through the soil had to be determined by comparing the amounts lost from three or four similarly prepared pots not planted to a crop.

The culture pots thus constructed were entirely satisfactory, and the plants grown in them were very vigorous and looked very healthy. They

compared very favorably with plants of the same variety in the field. Marquis wheat, the variety used here, grows to a height varying from 2.4 to 4 feet in the field. Those in the greenhouse varied from 3.2 to 3.8 feet. The heads were well formed, and varied in length from 2.7 inches to 3.8 inches, while in the field the variation is from 2.5 to 4 inches. In one respect, the plants in the field differed markedly from those in the greenhouse. In the field, Marquis averages 3 tillers to a plant, and all are likely to produce heads. But in the greenhouse, only 16 plants out of the 420 had tillers, and from these only one head was formed. The short duration of light, and its low intensity during the winter months when the experiment was conducted, probably account for the condition mentioned above (1). Two photographs of the plants were taken, one at an early stage of development, and the other at harvest time. These are shown in figures 1 and 2.

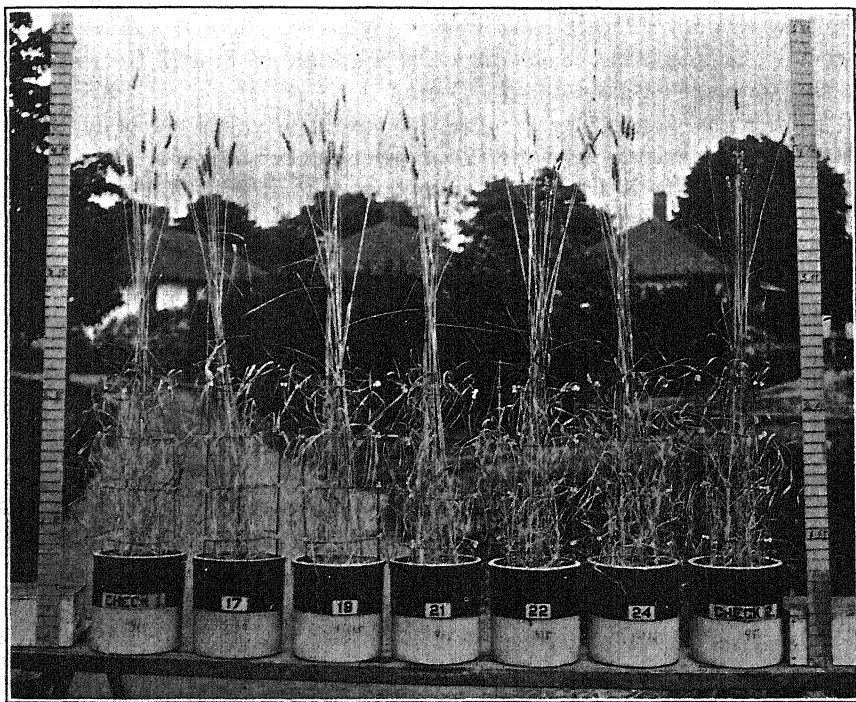


FIG. 2. Condition of plants at harvest time.

Comparison of an average field plant with an average plant grown in the greenhouse shows a close resemblance in all respects, except for the number of tillers. The field plants yield more grain because of the number of tillers. In table I are shown the field records of "Marquis wheat per

three square yards" grown in Wisconsin and supplied to us by Professor ARNY.

TABLE I
MARQUIS WHEAT PER THREE SQUARE YARDS

PLOT NUMBER	NUMBER OF PLANTS	NUMBER OF CULMS	WEIGHT OF STRAW	WEIGHT OF GRAIN
			gm.	gm.
29	154	379	481.8	237.5
34	172	337	444.8	241.5
39	199	390	475.0	250.3
Average	175	369	467.2	243.1

In a letter dated November 15, 1920, Professor ARNY states that "the plants were pulled and separated as nearly as possible and the above data taken." He also states that "when plants are grown in drill rows it is not possible to make an absolute separation."

Taking the averages in the table, the weight of culms per plant is 2.66 grams, and the average weight of grain per plant is 1.39 gram. The weight of straw per culm is 1.27 gram, and the weight of grain per culm, 0.656 gram. The average weight of culm calculated from our data is 1.44 gram, while for the 24 plants in the two check pots it is 1.26 gram. The average weight of grain per culm calculated from our data is 0.671 gram, while for the checks it is 0.598 gram. We may therefore safely conclude that the culture pots were suitably constructed for the growth of Marquis wheat in the greenhouse.

THE SOIL.—This was obtained from the field where the outdoor experiment was conducted. It was a composite sample of surface soil taken from a depth of not more than four inches from various parts of the field. In technical terms this soil is called a fine sandy loam of the Miami Series. At the time of the experiment, the moisture content of the soil was 1.11 per cent., the water-holding capacity 38.29 per cent., and the organic matter 4.95 per cent. These data enabled me to determine the amount of water to add to the pots to allow for excellent growth of the plants.

FERTILIZERS.—The three salts commonly used in the United States for fertilizer mixtures are $\text{Ca}(\text{H}_2\text{PO}_4)_2$, NaNO_3 , and K_2SO_4 . An analysis of each of the fertilizer salts was necessary in order to determine the exact amount of active ingredients present. Potassium sulphate contained 47.79 per cent. of K_2O , sodium nitrate 15.1 per cent. of nitrogen, and calcium acid phosphate 16.5 per cent. of available P_2O_5 .

In the selection of fertilizer compounds, it is obvious that they should contain all of the essential elements, and that the compounds should be

compatible. In the sodium salt, we are introducing the Na ion which is not classed among those essential. The essential Mg ion is not present in any compound. It is assumed here that the Mg ion present in the soil is sufficiently abundant and need not be added as a commercial fertilizer. However, if water culture experiments were being planned, Mg would be necessary, and modification of the experiment would be advisable.

LIVINGSTON and TOTTINGHAM (9) have shown that there are differences in the growth of plants, depending on which basic and acidic radicals are united.

They have shown, in a preliminary test, that when KNO_3 is substituted for $\text{Ca}(\text{NO}_3)_2$, and when the H_2PO_4 ion is united with the Ca ion, better growth results. They suggested a thorough testing of the six possible combinations of the six essential elements of the ions Ca, Mg, NO_3 , H_2PO_4 , K, and SO_4 .

FERTILIZER MIXTURES.—After studying the literature on fertilizer for wheat, it was decided to make the application at the rate of 100 pounds of active ingredients to the acre. Regardless of the filler in the fertilizer and regardless of the ratio of the various ingredients to each other, this means that the total weight of P_2O_5 , K_2O and N in the mixture must always be 100. Calculation showed that the above application per acre would require, in the case of each pot of 2-gallon capacity, 320 milligrams of active ingredients. The actual amounts of the different ingredients for each pot or culture was determined in the following manner: The total weight (320 milligrams) was distributed among the three salts in such a manner as always to sum up to ten, since it had been arbitrarily determined that the proportions were to vary in ten per cent. increments. With three variables under consideration (N, P_2O_5 , and K_2O) varying by increments of ten per cent., there are thirty-six possible combinations or ratios. For further details concerning the method of calculating the various ratios see TOTTINGHAM (12). The soil and fertilizers required for each pot were thoroughly mixed in the manner suggested by TRUOG (14). Besides the thirty-six cultures called for, twelve others were set up. Two were used as controls, four with an application of acid phosphate alone, three with sodium nitrate alone, and three with potassium sulphate alone. The quantities in grams in each of the ingredients, and for each fertilizer per pot are noted in table II.

SEEDS.—The seedlings for this experiment were raised from pure line Marquis wheat seeds supplied by W. E. TOTTINGHAM, of the Wisconsin Station, from the 1917 crop. The seeds were germinated on a paraffined mosquito netting over a dish of weak nutrient solution. When about six to nine centimeters tall those measuring 8 centimeters were transplanted into the pots, the seed part being buried to a depth of three-quarters of an inch. Each pot was planted to 12 seedlings. A recent study (13) has shown that

TABLE II
THE QUANTITIES OF P_2O_5 , K_2O AND N PER POT

Pot number	ACID PHOSPHATE 16.5 PER CENT. P_2O_5		SODIUM NITRATE 15.1 PER CENT. N		POTASSIUM SULPHATE 47.79 PER CENT. K_2O		TOTAL	
	P_2O_5 per pot	Fertilizer per pot	Nitrogen per pot	Fertilizer per pot	K_2O per pot	Fertilizer per pot	P_2O_5 + N + K_2O per pot	P_2O_5 + N per acre
1, 1, 8	gm. 0.032	gm. 0.194	gm. 0.032	gm. 0.212	gm. 0.256	gm. 0.536	gm. 0.32	lbs. 100
1, 2, 7	0.032	0.194	0.064	0.424	0.224	0.469	0.32	100
1, 3, 6	0.032	0.194	0.096	0.636	0.192	0.402	0.32	100
1, 4, 5	0.032	0.194	0.128	0.848	0.160	0.335	0.32	100
1, 5, 4	0.032	0.194	0.160	1.060	0.128	0.268	0.32	100
1, 6, 3	0.032	0.194	0.192	1.272	0.096	0.201	0.32	100
1, 7, 2	0.032	0.194	0.224	1.484	0.064	0.134	0.32	100
1, 8, 1	0.032	0.194	0.256	1.696	0.032	0.067	0.32	100
2, 1, 7	0.064	0.388	0.032	0.212	0.224	0.469	0.32	100
2, 2, 6	0.064	0.388	0.064	0.424	0.192	0.402	0.32	100
2, 3, 5	0.064	0.388	0.096	0.636	0.160	0.335	0.32	100
2, 4, 4	0.064	0.388	0.128	0.848	0.128	0.268	0.32	100
2, 5, 3	0.064	0.388	0.160	1.060	0.096	0.201	0.32	100
2, 6, 2	0.064	0.388	0.192	1.272	0.064	0.134	0.32	100
2, 7, 1	0.064	0.388	0.224	1.484	0.032	0.067	0.32	100
3, 1, 6	0.096	0.582	0.032	0.212	0.192	0.402	0.32	100
3, 2, 5	0.096	0.582	0.064	0.424	0.160	0.335	0.32	100
3, 3, 4	0.096	0.582	0.096	0.636	0.128	0.268	0.32	100
3, 4, 3	0.096	0.582	0.128	0.848	0.096	0.201	0.32	100
3, 5, 2	0.096	0.582	0.160	1.060	0.064	0.134	0.32	100
3, 6, 1	0.096	0.582	0.192	1.272	0.032	0.067	0.32	100

TABLE II—(Continued)
THE QUANTITIES OF P_2O_5 , K_2O AND N PER POT

Pot number	ACID PHOSPHATE 16.5 PER CENT. P_2O_5		SODIUM NITRATE 15.1 PER CENT. N		POTASSIUM SULPHATE 47.79 PER CENT. K_2O		TOTAL	
	P_2O_5 per pot	Fertilizer per pot	Nitrogen per pot	Fertilizer per pot	K_2O per pot	Fertilizer per pot	P_2O_5 + N + K_2O per pot	P_2O_5 + N + K_2O per acre
4, 1, 5	gm. 0.128	gm. 0.776	gm. 0.032	gm. 0.212	gm. 0.160	gm. 0.335	gm. 0.32	lbs. 100
4, 2, 4	0.128	0.776	0.064	0.424	0.128	0.268	0.32	100
4, 3, 3	0.128	0.776	0.096	0.636	0.096	0.201	0.32	100
4, 4, 2	0.128	0.776	0.128	0.848	0.064	0.134	0.32	100
4, 5, 1	0.128	0.776	0.160	1.060	0.032	0.067	0.32	100
5, 1, 4	0.160	0.970	0.032	0.212	0.128	0.268	0.32	100
5, 2, 3	0.160	0.970	0.064	0.424	0.096	0.201	0.32	100
5, 3, 2	0.160	0.970	0.096	0.636	0.064	0.134	0.32	100
5, 4, 1	0.160	0.970	0.128	0.848	0.032	0.067	0.32	100
6, 1, 3	0.192	1.164	0.032	0.212	0.096	0.201	0.32	100
6, 2, 2	0.192	1.164	0.064	0.424	0.064	0.134	0.32	100
6, 3, 1	0.192	1.164	0.096	0.636	0.032	0.067	0.32	100
7, 1, 2	0.234	1.358	0.032	0.212	0.064	0.134	0.32	100
7, 2, 1	0.234	1.358	0.064	0.424	0.032	0.067	0.32	100
8, 1, 1	0.256	1.552	0.032	0.212	0.032	0.067	0.32	100
Single fertilizers		1.552 0.776 0.388 0.194		1.696 0.848 0.424		0.536 0.268 0.134		
Check number 1, no fertilizer								Check number 2, no fertilizer

uniform initial seed weight reduces variability, and that selection of young seedlings for uniformity of height exerts a similar influence, although probably less pronounced. Each culture was brought to a water content equal to 60 per cent. of its water-holding capacity. A layer of gravel covered the soil surface to reduce the surface loss of moisture. Each pot was then weighed and the weight recorded on the pot. This is necessary if one desires to know the water loss for definite periods of time.

ENVIRONMENTAL CONDITIONS.—The experiment was conducted in the greenhouse starting on December 16th and ending at harvest time, June 3rd. The cultures were placed on a large rotating table, 8 feet in diameter. There were too many pots to put in a single row so they were arranged in three rows as near the margin of the table as possible. The position of the cultures was changed from one row to another every three days. Some studies were made to determine the effect of the position of a culture on transpiration. It was found that cultures on the outer row transpired more water than those on the inner row and the middle row, while those on the inner row transpired more than those on the middle row. This was true for the period of this test or for about two weeks while the cultures were in the three rows. After this the cultures were set in two rows and the transpiration from them was not appreciably affected by whether the culture was in one or the other row. There were a few exceptions to this general rule and these were due to the fact that in one row there happened to be a larger proportion of cultures that were transpiring more heavily, and had so transpired from the beginning. In these tests on transpiration, it was further found that some cultures always transpired heavily no matter what row they were in. A few other cultures were low transpirers throughout the experiment.

At the start of the experiment the light intensity was probably not as conducive to good growth as might be desired, yet the plants did not appear to suffer. Shortly after the experiment was started, the days began to lengthen. When light became too intense during the latter part of the experimental period, cheese cloth was used to reduce the intensity, or the glass of the greenhouse was whitewashed. The temperature of the greenhouse for a period of nine weeks during the winter months was kept close to 70° F. by thermostatic regulation. Later in the season, with the steam off and the ventilators open, outdoor conditions were the rule, except at night when the ventilators were closed. During this period of five weeks, there were two low readings, and a few high ones (80° to 90° F.) at mid-day. During the last ten weeks, the temperature variations were more frequent and of longer duration. The plants, however, suffered at no time. The records show that 83 per cent. of the possible optimum range of tem-

perature for wheat was attained. This may be considered a very good percentage. A summary of the thermographic records is shown in table III.

TABLE III

SUMMARY OF THE THERMOGRAPHIC RECORDS FOR THE PERIOD OF THE EXPERIMENT

TWO-WEEK PERIODS	MAXIMUM	DURATION	MINIMUM	DURATION	DURATION ABOVE 70° F.	DURATION BELOW 55° F.	TOTAL DURATION 55°-70°	TOTAL DURATION 55°-70°
	°F	Hours	°F	Hours	Hours	Hours	Hours	Per cent.
Dec. 16-29 '18	87	0.5	60	0.5	15		321	95.5
Dec. 30-Jan. 12...	87	0.5	56	0.5	18		318	94.6
Jan. 13-Jan. 26...	92	0.5	62	0.5	16		302	95.2
Jan. 27-Feb. 9...	78	0.5	49	0.5	12	1	323	96.0
Feb. 10-Feb. 23...	82	1.0	54	0.5	17	1	318	94.6
Feb. 24-Mar. 9...	75	0.5	42	0.5	20	41	275	81.9
Mar. 10-Mar. 23...	88	1.0	49	0.5	37	6	293	87.2
Mar. 24-Apr. 6...	97	0.5	38	0.5	76	49	211	62.8
Apr. 7-Apr. 20...	89	0.5	48	1.0	27	35	274	81.5
Apr. 21-May 4...	85	1.0	48	1.0	23	21	291	86.9
May 5-May 18...	94	2.0	45	1.5	74	45	217	64.5
May 19-June 1...	100*	1.5*	50	5.0	116	38	182	54.1
Total		10		12.5	451	197	3333	82.9

* Reached 100° at noon on six consecutive days for a period of 15 minutes each time.

The optimum range for wheat was taken as 55°-70° F., the range determined by HUTCHESON and QUANTZ (5) in their study on greenhouse temperatures as affecting the growth of small grains. The air temperature records were taken from a thermograph, shaded from the sun in a temperature cage.

That the air moisture condition in the greenhouse is an important factor in plant growth is well recognized, and a thorough wetting down of soil, benches, and walks daily is the usual practice. A too moist condition, however, is conducive to attacks of mildew, while a very dry greenhouse is unsuitable for plants, causing a drying of leaves, followed usually by the death of the plant, even though the roots are in moist soil or even in water cultures. The moisture condition of the air is usually given in terms of relative humidity, but unless the temperature is stated also the picture of the conditions is inadequate. The same percentages of relative humidity have very different meanings when the temperatures differ widely. Take, for example, an illustration from the present study. In the early part of the experiment, December 23rd, at 8 o'clock A. M., the relative humidity was 57 per cent. On June 7th at the same time of day, the relative humid-

ity was the same, but in the first case the temperature was 69° , while in the second it was 75° . The air moisture factor in the one case was 9.23 mm.; in the other, 12.65 mm. The relative humidity was the same, but the air moisture factors differed by 3.42 mm. Like relative humidities do not therefore present similar pictures of the air moisture condition except when the temperatures are the same. It is preferable, therefore, to determine the air moisture conditions from records of the evaporating power of the air as shown by a standard evaporating surface, such as the porous clay spheres, or Livingston atmometers.

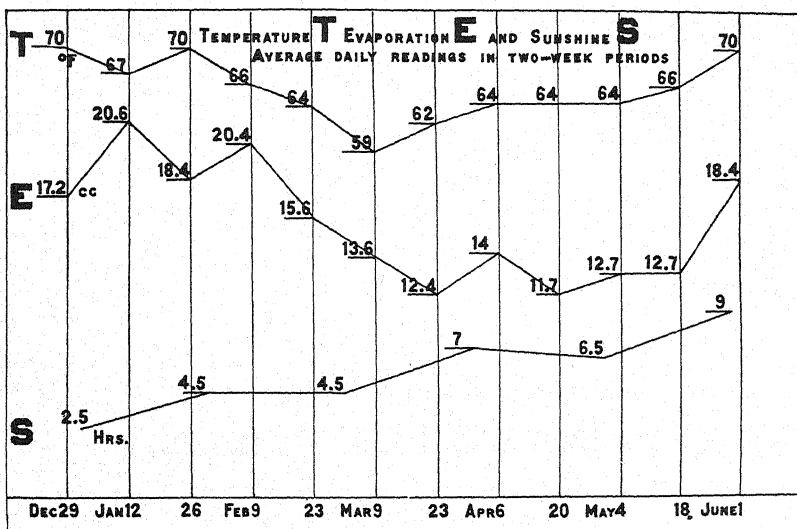


FIG. 3. Mean daily temperature, T, in degrees F.; mean daily evaporation, E, in cc.; and mean daily duration of sunshine, S, in hours in two-week periods. S is given for outdoors, and this condition is much reduced inside the greenhouse.

The air moisture condition, or atmometric index, was derived from records obtained from two standard white spherical atmometers kept on the rotating tables during the experiment. Humidity percentages were calculated three times daily (8 A. M., 12 M., and 6 P. M.) from results obtained by the sling psychrometer. The hygrograph did not prove satisfactory and was discarded. The sling psychrometer was mounted on a board, the bulb of the wet thermometer resting over a hole in the board, which allowed free circulation of air over the wet bulb. When a reading was desired, a current of air from an electric fan was directed at the apparatus, the reading made, and humidities calculated from the psychrometric tables. Curves

of temperature, evaporation, and sunshine duration are given in fig. 3. The results in table III are given in two-week periods.

Some mutual shading of the well-grown plants could not be avoided. An attempt was made, however, to minimize the error arising from this condition by changing the position of the cultures on the rotating table at the end of every three-day period, at the time when distilled water was added. Plants from the inner row were changed to the outer row and *vice versa*.

This consideration of the environment is by no means complete, nor is one to infer from the separate discussion of the individual factors already made that these act upon the plants singly. The influences acting upon the plant are the resultant of all factors. This is the reason why it has been so difficult to evaluate the climatic complex.

FUMIGATION OF GREENHOUSE.—On December 10th, just before the experiment was started, it was observed that mildew was appearing on wheat plants in other portions of the greenhouse. Fumigation was accomplished by painting the steam pipes that night with a sulphur paste. By carefully controlling the humidity conditions in the greenhouse, and by keeping the temperature near that suitable for the growth of wheat (60° – 70° F. during the day, and 50° – 60° F. during the night) we were not troubled again during the period of the experiment.

In early March thrips and lice appeared and these were controlled by fumigation over night with "Black Leaf 40" at the rate of one ounce for every 1,000 cubic feet of space. The fumigation was repeated the following morning. This controlled the insects for a time but they appeared again in April. On the 3rd of April, the third and last fumigation was made and this proved effective for the rest of the experimental period. These various fumigations had no deleterious effects on the wheat at any time.

This statement of the experimental methods, although too long, is desirable, for much of the difference in results obtained by various workers might be eliminated if the description of conditions were sufficiently detailed to allow real duplication of conditions.

Discussion of results

THREE SALT CULTURES

This experiment deals with the growth of Marquis wheat in soil (pot cultures) in the greenhouse during the winter of 1918–19. The plants were grown to maturity, were healthy and robust, and compared exceedingly well with plants grown in the field. The well known triangle system for determining the best salt ratio for optimum yields was tried out. The actual gain or loss in any particular culture is figured on the basis of check as unity, and is obtained by dividing the culture yield by the check yield.¹

¹ The check yields are the yields from two cultures of twelve plants each, in fertile soil that had received no special fertilizer treatment.

The final results of the yield of grain and straw are given in tables IV and V. Special attention is called to column 8 in table IV which shows the average weight of grain relative to check as unity. It is from this column that a picture of the differences in yield for the various cultures can be drawn. The same is true for column 7 in table V in regard to straw yield. The last column in each table indicates the rank of the low and high yielding cultures. The nine high yielding cultures are indicated by an H preceded by the figure indicating the rank. The nine cultures showing the lowest yields are indicated by an L preceded by the figure indicating the rank.

The high and low yields are plotted on the triangle in figure 4. With very few exceptions, the low grain yields are grouped in that angle of the triangle which represents high ratios of K_2O . The same is true with respect to straw yields. High ratios of K_2O are not conducive to good growth. A further proof that high applications of K_2O are bad is found in the results obtained where this single salt is used. When 536 milligrams are added per pot (at the rate of 168 pounds per acre), the grain yield is reduced 7 per cent. and the straw yield by 18 per cent. when compared with the checks. When 268 milligrams are added per pot (application at the rate of 84 pounds per acre), the yield of grain is increased by 6 per cent. and the yield of straw by only 1 per cent. At the rate of 42 pounds per acre (134 milligrams per pot), the results are again negative, the grain yield being reduced 8 per cent. and the straw yield 2 per cent.

In regard to the high yields, the results as shown on the triangle are not what might have been expected. From *a priori* reasoning a single best culture should be found, and grading from this in all directions should be found the succeeding high yielding cultures. In this particular experiment there are two high points. Many workers have found two high regions and two low regions. No explanations have been offered, but it is evident that the centers of groups of good cultures might be just off the particular triangle studied, while a few cultures forming the outer margin of high yielding regions would enter the triangle. This explanation, however, is not adequate in the present case, as it is very apparent that three or four of the good cultures are scattered in widely different parts of the triangle with poor cultures their immediate neighbors.

A glance at the yield figures of tables IV and V shows one exceptionally high culture, and it could be argued that it gave an abnormally high yield, and should rightly be eliminated from consideration. Two or three other cultures could be eliminated for various reasons, but in this process of elimination where shall one stop? If we eliminate the cultures at the upper and lower right corners of the triangle, and the two in the medium lower right angle, there would be left five cultures of about the same grade of

TABLE IV

GRAIN YIELD DATA FOR MARQUIS WHEAT IN GREENHOUSE, DECEMBER 16, 1918-JUNE 9, 1919

CULTURE NUMBER	WEIGHT OF HEADS WITH GRAIN, ALL PLANTS	AVERAGE WEIGHT OF HEADS WITH GRAIN	TOTAL NUMBER OF GRAINS	AVERAGE NUMBER OF GRAINS PER PLANT	TOTAL WEIGHT OF GRAIN PER PLANT	AVERAGE WEIGHT OF GRAIN PER PLANT	AVERAGE WEIGHT OF GRAIN RELATIVE TO CHECK	PER CENT. OF GRAIN PER PLANT	WEIGHT OF 100 GRAINS	PER CENT. INCREASE GRAIN WEIGHT OVER CHECK	HIGH YIELDING AND LOW YIELDING CULTURES
	gm.	gm.			gm.	gm.	gm.		gm.	gm.	
1, 8	11.1	0.925	243	20.3	8.50	0.708	1.20	76.5	3.50	20.0	1L
2, 7	9.2	0.767	213	17.9	6.89	0.574	0.96	75.8	3.23	4.0	2L
3, 6	9.1	0.758	194	16.2	7.00	0.583	0.98	76.9	3.61	2.0	
4, 5	10.2	0.927	222	20.2	7.90	0.718	1.20	77.5	3.56	20.0	
5, 4	9.2	0.767	196	16.3	7.01	0.584	0.98	76.1	3.58	2.0	3L
6, 3	10.4	0.867	225	18.8	8.19	0.683	1.14	78.8	3.64	14.0	
7, 2	11.3	0.942	237	19.8	8.80	0.733	1.23	77.8	3.71	23.0	9H
8, 1	12.0	1.000	256	21.3	9.46	0.788	1.32	78.8	3.70	32.0	3H
9, 1	9.2	0.836	203	18.5	7.10	0.645	1.08	77.2	3.50	8.0	4L
1, 7	9.8	0.754	214	16.0	7.65	0.589	0.98	78.1	3.57	2.0	
2, 6	10.3	0.858	229	19.1	8.09	0.674	1.13	78.6	3.53	13.0	1H
3, 4	14.3	1.300	286	26.0	10.90	0.991	1.66	76.3	3.81	66.0	
5, 3	10.2	0.850	225	18.8	7.99	0.666	1.11	78.4	3.55	11.0	5L
6, 2	9.4	0.783	206	17.2	7.28	0.607	1.01	77.5	3.53	1.0	
7, 1	8.9	0.809	199	18.1	7.01	0.637	1.06	78.7	3.52	6.0	7L
1, 6	9.5	0.792	208	17.3	7.47	0.624	1.05	78.8	3.59	4.0	8L
2, 5	9.8	0.817	216	18.0	7.47	0.624	1.04	76.4	3.46	4.0	
3, 4	10.2	0.850	232	19.3	8.14	0.678	1.13	79.7	3.51	13.0	
4, 3	11.1	0.925	229	19.1	8.49	0.708	1.18	76.5	3.70	18.0	9L
5, 2	10.4	0.800	233	18.0	8.08	0.622	1.04	77.8	3.46	4.0	2H
6, 1	12.7	1.058	265	22.1	9.82	0.818	1.37	77.3	3.72	37.0	6H
1, 5	11.6	0.967	247	20.6	9.17	0.764	1.28	79.0	3.71	28.0	7H
2, 4	11.2	0.953	242	20.2	9.01	0.751	1.26	80.5	3.72	26.0	
3, 3	11.0	0.917	235	19.6	8.56	0.713	1.19	77.7	3.65	19.0	

GRAIN YIELD DATA FOR MARQUIS WHEAT IN GREENHOUSE, DECEMBER 16, 1918-JUNE 9, 1919

TABLE IV—(Continued)

CULTURE NUMBER	WEIGHT OF HEADS WITH GRAIN, ALL PLANTS	AVERAGE WEIGHT OF HEADS WITH GRAIN	TOTAL NUMBER OF GRAINS	AVERAGE NUMBER OF GRAINS PER PLANT	TOTAL WEIGHT OF GRAIN PER PLANT	AVERAGE WEIGHT OF GRAIN PER PLANT	AVERAGE WEIGHT OF GRAIN RELATIVE TO CHECK	PER CENT. OF GRAIN PER PLANT	WEIGHT OF 100 GRAINS	PER CENT. INCREASE GRAIN WEIGHT OVER CHECK	HIGH YIELDING AND LOW YIELDING CULTURES
	gm.	gm.			gm.	gm.	gm.		gm.	gm.	
4, 1	10.2	0.850	213	17.8	7.79	0.649	1.09	76.4	3.66	9.0	6L
5, 1, 4	9.6	0.800	203	16.9	7.29	0.608	1.02	76.0	3.59	2.0	8H
5, 2, 3	10.5	0.875	230	19.2	8.13	0.678	1.13	77.5	3.53	13.0	5H
5, 3, 2	11.4	0.950	234	19.5	8.78	0.732	1.23	77.1	3.75	23.0	4H
5, 4, 1	10.7	0.892	224	18.7	8.38	0.698	1.17	78.2	3.74	17.0	
6, 1, 3	10.2	0.850	218	18.2	7.94	0.662	1.11	77.9	3.64	11.0	
6, 2, 2	11.7	0.975	250	20.8	9.13	0.761	1.28	78.1	3.65	28.0	
6, 3, 1	10.1	0.842	226	18.9	7.87	0.656	1.09	77.9	3.48	9.0	
7, 1, 2	10.5	0.875	227	18.9	8.19	0.683	1.14	78.1	3.61	14.0	
7, 2, 1	10.6	0.883	234	19.5	8.23	0.686	1.15	77.7	3.52	15.0	
8, 1, 1	12.0	1.000	250	20.8	9.28	0.773	1.29	77.3	3.71	29.0	
Check 1	9.7	0.808	208	16.9	7.65	0.600	0.00	00.0	0.00	0.0	
Check 2	8.9	0.742	197	16.4	6.82	0.598	1.00	77.4	3.71	0.0	
P ₂ O ₅ 1	10.4	0.867	233	19.5	8.21	0.684	1.14	78.9	3.52	14.0	
P ₂ O ₅ 2	10.0	0.769	215	16.6	7.71	0.593	0.99	77.1	3.59	1.0	
P ₂ O ₅ 3	10.0	0.833	215	16.6	7.79	0.649	1.09	77.9	3.62	9.0	
P ₂ O ₅ 4	8.4	0.700	179	14.9	6.43	0.536	0.90	76.6	3.78	—10.0	
N 1	11.5	0.959	236	19.7	8.92	0.743	1.24	77.5	3.73	24.0	
N 2	12.6	1.050	266	22.2	9.91	0.826	1.38	78.7	3.73	38.0	
N 3	8.5	0.709	188	15.7	6.52	0.543	0.91	76.6	3.47	—9.0	
K ₂ O 1	8.7	0.725	189	15.8	6.66	0.555	0.93	76.6	3.54	—7.0	
K ₂ O 2	10.5	0.808	230	17.7	8.23	0.633	1.06	78.3	3.58	6.0	
K ₂ O 3	8.5	0.654	189	14.6	6.56	0.547	0.92	83.7	3.47	—8.0	

TABLE V
STRAW YIELD DATA FOR MARQUIS WHEAT IN THE GREENHOUSE, DECEMBER 16, 1918-JUNE 9, 1919

CULTURE NUMBER	AVERAGE LENGTH OF PLANTS	AVERAGE LENGTH OF HEADS	AVERAGE LENGTH OF STRAW	WEIGHT OF STRAW ALL PLANTS	AVERAGE WEIGHT OF STRAW PER PLANT	WEIGHT OF STRAW RELATIVE TO CHECKS	PER CENT. INCREASE OR DECREASE OVER CHECK	HIGH YIELDING AND LOW YIELDING CULTURES
	cm.	cm.	cm.	gm.	gm.	gm.		
1, 1, 8	104.3	7.3	97.0	17.2	1.43	1.13	13.0	1L
1, 2, 7	95.3	7.3	88.0	17.3	1.19	0.94	- 6.0	7L
1, 3, 6	103.1	6.9	96.2	15.3	1.28	1.02	2.0	
1, 4, 5	106.3	7.6	98.7	16.6	1.51	1.19	19.0	
1, 5, 4	98.6	7.3	91.3	14.4	1.20	0.95	- 5.0	3L
1, 6, 3	104.3	8.1	96.2	16.9	1.41	1.12	12.0	
1, 7, 2	99.6	7.5	92.1	16.6	1.38	1.09	9.0	
1, 8, 1	109.2	8.5	100.7	19.4	1.62	1.28	28.0	6H
2, 1, 7	102.1	8.6	93.5	16.4	1.49	1.18	18.0	
2, 2, 6	103.3	7.1	96.2	15.5	1.19	0.94	- 6.0	2L
2, 4, 4	105.6	8.3	97.3	16.6	1.38	1.09	9.0	1H
2, 5, 3	114.1	9.5	104.6	21.1	1.92	1.52	52.0	
2, 6, 2	106.4	8.4	98.0	18.0	1.50	1.19	19.0	
2, 7, 1	104.2	7.8	96.4	16.6	1.38	1.09	9.0	5L
3, 1, 6	103.9	7.0	96.9	13.9	1.26	1.00	0.0	4L
3, 2, 5	103.8	6.8	97.0	14.6	1.22	0.97	- 3.0	
3, 3, 4	102.8	8.0	93.8	16.7	1.39	1.09	9.0	
3, 4, 3	108.2	7.5	100.7	16.8	1.40	1.09	9.0	
3, 5, 2	110.7	9.1	101.6	18.3	1.53	1.21	21.0	2H
3, 6, 1	105.7	8.9	96.8	18.8	1.45	1.15	15.0	8H
4, 1, 5	111.0	8.3	102.7	20.5	1.71	1.36	36.0	
4, 2, 4	111.4	8.1	103.3	18.7	1.56	1.24	24.0	
4, 3, 3	113.7	7.7	106.0	19.7	1.64	1.30	30.0	4H
4, 4, 2	107.9	8.4	99.5	17.6	1.47	1.17	17.0	

TABLE V—(Continued)

STRAW YIELD DATA FOR MARQUIS WHEAT IN THE GREENHOUSE, DECEMBER 16, 1918–JUNE 9, 1919

CULTURE NUMBER	AVERAGE LENGTH OF PLANTS	AVERAGE LENGTH OF HEADS	AVERAGE LENGTH OF STRAW	WEIGHT OF STRAW ALL PLANTS	AVERAGE WEIGHT OF STRAW PER PLANT	WEIGHT OF STRAW RELATIVE TO CHECKS	PER CENT. INCREASE OR DECREASE OVER CHECK	HIGH YIELDING AND LOW YIELDING CULTURES
4, 5, 1	cm. 107.6	cm. 8.7	cm. 98.9	gm. 17.1	gm. 1.43	gm. 1.14	14.0	6L
5, 1, 4	104.3	6.9	97.4	15.3	1.28	1.02	2.0	9H
5, 2, 3	109.6	8.6	101.0	18.2	1.52	1.21	21.0	3H
5, 3, 2	109.6	8.8	100.8	20.1	1.68	1.33	33.0	
5, 4, 1	106.9	8.0	98.9	17.7	1.48	1.17	17.0	
6, 1, 3	106.9	7.8	99.1	16.5	1.38	1.09	9.0	
6, 2, 2	111.7	8.0	103.7	19.5	1.63	1.30	30.0	5H
6, 3, 1	109.6	7.4	102.2	15.8	1.32	1.05	5.0	8L
7, 1, 2	105.6	7.9	97.7	16.5	1.38	1.09	9.0	9L
7, 2, 1	104.6	8.5	96.1	16.2	1.35	1.07	7.0	7H
8, 1, 1	109.9	8.7	101.2	19.3	1.61	1.28	28.0	
Check 1	106.4	7.4	98.0	15.7	1.32	1.00	0.0	
Check 2	100.5	7.7	92.8	14.4	1.20	1.00	0.0	
P ₂ O ₅ 1	104.9	7.8	97.1	16.5	1.37	1.09	0.0	
P ₂ O ₅ 2	112.4	7.9	104.5	15.6	1.20	0.95	- 5.0	
P ₂ O ₅ 3	104.6	9.1	95.5	15.4	1.28	1.02	2.0	
P ₂ O ₅ 4	96.6	6.8	89.8	12.9	1.08	0.86	- 14.0	
N 1	106.7	8.4	98.3	17.6	1.48	1.18	18.0	
N 2	107.0	8.3	98.7	17.9	1.49	1.19	19.0	
N 3	100.6	7.3	93.3	13.0	1.08	0.86	- 14.0	
K ₂ O 1	98.3	7.2	91.1	12.4	1.03	0.82	- 18.0	
K ₂ O 2	102.9	8.4	94.5	16.5	1.27	1.01	1.0	
K ₂ O 3	99.3	7.1	92.2	13.1	1.01	0.80	- 20.0	

value near the upper middle region. This shows that there is a demand for acid phosphate, and is in accord with the general field experience that small grains require considerable phosphoric acid. Perhaps it may be safe to say that phosphoric acid is required more than nitrogen since there are more high cultures nearer that angle than the nitrogen angle. This reasoning, however, is not satisfying.

It is not beyond reason to try to trace the condition found to variability of the individual plants. It has been suggested that variability of plants in water culture is so great that it may obscure the relation between the growth of plants and the physiological values of the solutions tested. The writer has demonstrated this in his own experiments several times in the last six or eight years. The same might be true of plants in soil cultures, but it is not so strikingly evident in this experiment. The seeds used were from a pure line; they were selected carefully according to standard rules, were germinated under controlled conditions, and the test plants selected for uniform height and growth from a large group. All were planted at the same depth in the soil and given the same care. At harvest time, the height as well as the length of head of every plant in each culture was measured. In addition, the following data were also collected for each culture: Total weight of heads and grain, total weight of grain, total number of grains, weight of 100 grains, and total weight of straw.

Variability in the height of the plants in a culture was quite small. The coefficients of variability for all of the cultures were determined and the average was 8.2 per cent. The highest coefficient in the whole group was 13.3 per cent. This is not considered high, judging from the records which have been obtained from various experiments both in the greenhouse and in the field under conditions where great variability was sought, and where, by controlling conditions, variability could be held down to a low per cent. There was considerable variability between cultures; but within a single culture there was very little individual variability. By further study it was found that among the 9 best cultures there was a real difference only when the best and the poorest in the group were compared. The odds of 144 to 1 showed a real difference between the poorest and the best in the group of 9 best cultures. The others did not show any significant difference. LOVE's modification of Student's Method was used in these computations. When any culture in the group of 9 best was compared with any in the group of the 9 poorest cultures, the differences were significant also. Finally as to the check cultures, two in number, it can be said that they were similar, there being no significant differences between them as far as the height of the plants is concerned. Calculated by Student's method, the odds were merely 10.9 to 1 and by BESSEL's method, 13.5 to 1. The above facts indicate that variability within a culture is not sufficiently large to

explain the lack of satisfactory grouping of the high yields on the triangle. Furthermore, the data show that the salt ratios produced differences in cultures throughout the triangle. The relation between the growth of plants and the physiological values of the solutions were not obscured, therefore, by variability. To charge the lack of grouping to variability in seedlings seems begging the question.

These considerations are based on records of the height of individual plants. That conclusions drawn from these height measurements are as reliable as those from individual dry weights or grain weights, which are more often offered, can be shown. The individual grain weight records were not gathered in this particular experiment, but those of the culture as a whole (12 plants) were obtained.

TABLE VI
CORRELATION BETWEEN HEIGHT OF PLANTS AND DRY WEIGHTS
Length of plants in cm., average of 12 plants

Weight of grain, average of 12 plants		96	99	100	102	103	104	105	107	109	111	112	114
	6.5	1	2	1									
	7.0	1	1	1	1	1	1						
	7.5				1	1	3	1					
	8.0					1	3	4	4	3			
	8.5						1		2		1		
	9.0								1	1	2		1
	9.5									2			
	10.0								1		1		
	11.0												1

The correlation between height of plants and weight of grain is shown in table VI. It shows a high positive correlation between the height of plants per culture and the weight of grain per culture. It is perfectly logical, therefore, to draw the conclusion that height of individual plants is correlated with their yield, and that the argument previously developed is just as important as if it had been based entirely on individual grain yields instead of height of individuals.

SCHREINER and SKINNER, who were originally responsible for the use of the triangular system for determining the salt requirements of agricultural crops in water cultures, early concluded that the ratios of salts might vary widely without appreciably affecting the yields. Others, working more

recently, though not using the triangular system in detail, have drawn the same conclusion, and might use the data here presented to support their own conclusions. Perhaps they would be correct. There is, however, another suggestion that might be presented and which may have more than a slight bearing on the question. Our agricultural crops have been for generations so modified by domestication that they have become accustomed to wide variation in environmental conditions and do not lend themselves to studies of this kind. Results might be very different with some wild variety raised in a restricted environment.

Finally, the observed facts may be due to the possible variability in the soil. The soil is a very complex medium for the growth of plants. In the field, there are many differences in localities within a few feet of each other. In this particular experiment every effort was made to prepare the soil so that uniform samples could be obtained for each culture. The soil was first air dried, then sifted, then redried, and broken up finally to pass through a 4 mm. mesh sieve. It was then stored in tight cans until needed. When ready to prepare the cultures, a definite amount of soil for each culture pot (6,400 grams) was placed on a clean paper on the table and thoroughly mixed with the sample of fertilizer for that particular culture. This mixing was done according to the plan suggested by TRUOG (14). It is not believed that the differences in the cultures were due to any possible difference in the soil used. That the differences are attributable to the fertilizer ratios is more logical. That the poor grouping of the best cultures could be traced to the soil used is not likely, considering the great care taken to make the soil uniform for all culture pots.

A duplicate of this experiment was started in September and was completed in March. There was excellent vegetation growth, but no heading out even after a period of one hundred and sixty-eight days. Light was considered the limiting factor, during the early period at least. After a careful study of the cultures as to general appearance, it was found that the same general conclusions would have to be drawn as in the first case. The experiment was terminated with no further collection of dry weight or other data.

In addition to the method illustrated in figure 4, there is still another way of calculating the effects of the fertilizer salts, *viz.*: by arranging the various cultures in groups. For example (see figure 5), the ten cultures in the top portion of the triangle represent cultures high in acid phosphate, while a similar number of cultures in the lower right portion represents cultures high in sodium nitrate. In the lower left portion of the triangle, the ten cultures are high in potassium sulphate. In the center of the triangle there are six cultures in which all three fertilizer ingredients are in equal or nearly equal amounts. When the average grain and straw weights

three, and showed that the poor cultures were well clustered in one region of the triangle.

The figures for grain yield are: Acid phosphate 16.1 per cent., sodium nitrate 17.6 per cent., center of the triangle 17.1 per cent., and potassium sulphate 9.6 per cent. The same order was followed when the straw yields were taken and calculated in like manner. This method does not give us results differing in any appreciable way from the other methods mentioned. This is a method that has been used by HARRIS (2), and in a modified form by Woods (15).

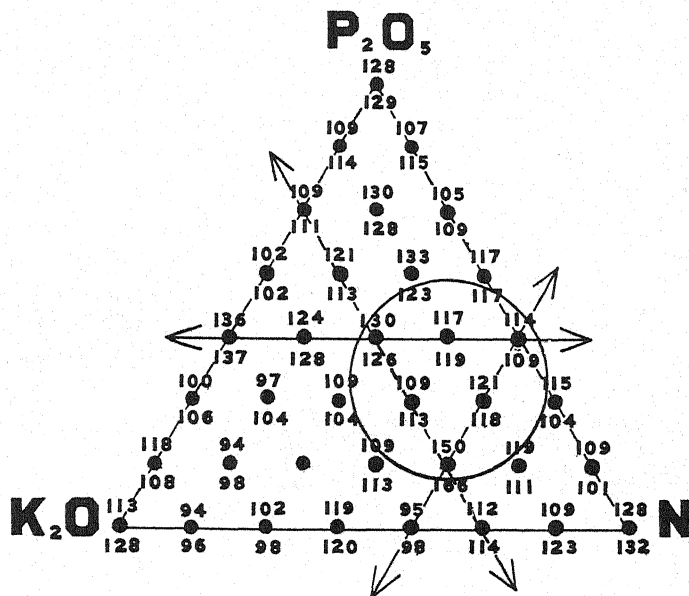


FIG. 6. Another method of grouping.

Finally, in figure 6 there is illustrated another method which shows that the concentration of the three fertilizer ingredients can vary quite a little and still not modify the yield to any appreciable extent. This method has been suggested by SCHREINER and SKINNER in the work referred to in the earlier part of this paper. In this calculation, the records for the cultures at the angle at the top, and that in the angle at the lower right were eliminated. This method in our opinion is no better than the others already discussed when one attempts to interpret the results.

It seems impossible to ascribe the lack of grouping of the best cultures to any other reason than that the plants used are accustomed to a variety of salt ratios and do not suffer appreciable differences in yield.

SINGLE SALT CULTURE

On referring to table I, the amounts of single fertilizer compounds per pot and per acre can be seen. At the end of tables IV and V are given the data for grain and straw yields from plants grown in pot cultures containing the amounts of the single salts indicated. Taken as a whole, the use of single fertilizers does not give as good results in soil cultures as the combination of three under the conditions of this experiment. Several other similar experiments have shown the same results. This had been previously shown for water cultures, and is now shown to be true for soil cultures even though such soil samples were from a field in high fertility. Of the ten single salt cultures, only two reached the value of the high group and these were the cultures where nitrogen was used alone. The per cent. gain in grain yield over the checks was 24 with an application of 1.696 gram per pot or on the basis of 528 pounds per acre; with an application of 0.848 gram per pot, equivalent to 264 pounds per acre, the gain was 38 per cent. The latter rate of application was better than the former as far as grain yield was concerned. The application of nitrogen benefited grain development, but vegetative growth as shown by straw yield did not equal any of the best nine cultures in the group containing the three fertilizer ingredients. As for $\text{Ca}(\text{H}_2\text{PO}_4)_2$ the best results obtained with four different rates of application was only 9 per cent. better than the checks. In this case the amount applied was 1.552 gram per pot, equal to 480 pounds per acre. The other three applications were at decreasing rates, the results showing inferiority to the yields of the controls. The yields of grain and straw when K_2SO_4 was applied at three different rates were either inferior to the controls, or equal to them, but not better. An application at the rate of 0.536 gram per pot, or 168 pounds per acre, was detrimental, cutting down the yield of both straw and grain. At one-fourth the application the yield was subnormal, while at one-half it was about the same as that of the controls.

Although the best yields are obtained with this soil where the three fertilizers are mixed, the increased cost of the mixture might make its use prohibitive. Only under such conditions would it be advisable to resort to the use of a single fertilizer. To determine whether a triple mixture with its increased yield over that of a single fertilizer costs less in the long run, a regular field experiment should be inaugurated.

CONDITION OF PLANTS

For the early period of growth the plants were normal in all respects and except for size there were no apparent differences between the cultures. The normal plant out in the field averages two or three tillers, while those grown in the greenhouse tillered sparingly. This was the only difference between the field and the greenhouse cultures. Only sixteen out of the

thirty-five different cultures showed tillers and none of these headed out. Culture 1, 1, 8, only an average yielder, produced 5 tillers. Only one culture (3, 3, 4), and this a poor yielder, possessed 4 tillers. There were four cultures that had three tillers each (1, 2, 7; 2, 1, 7; 2, 5, 3; and 6, 3, 1). All were poor yielders except 2, 5, 3, which was the best. The cultures possessing 2 tillers were two in number (2, 6, 2 and 4, 4, 2), merely average yielding cultures. There were eight cultures showing one tiller (1, 3, 6; 1, 5, 4; 1, 8, 1; 2, 4, 4; 3, 4, 3; 3, 6, 1; 5, 4, 1; and 8, 1, 1) varying from poor yielding cultures, through medium, to very high yielding ones. Under the conditions of this experiment one would not be justified in concluding that fertilizer combinations were even contributory agents in the formation of tillers.

On April 11 there was the first appearance of heads in cultures 3, 5, 2 and 6, 3, 1. On the 14th, heads appeared on cultures 1, 8, 1; 2, 1, 7; 1, 4, 5; and 8, 1, 1. On the 18th all the cultures with the exception of 1, 2, 7 and 1, 3, 6 had one or more plants showing heads. At this time out of a possible 418 plants there were 157 showing heads (37.5 per cent.). Five days later out of a possible 418 plants there were 363 showing heads (approximately 80 per cent.). The rest headed out a few days later. In approximately two weeks from the first appearance of a head they all headed. Blossoming started on April 11th and continued through and slightly beyond the period of heading out. The plants were harvested on the 9th of June after a growing period of 174 days. A good idea of the general appearance (color excepted) of the plants may be judged from the photographs, figures 1 and 2.

The plant measurements taken at harvest time, but not given here, comprise the following: Height of plant, length of head, and number of heads.

TRANSPIRATION

All the transpiration data include not only the loss of water from plants but also that from the surface soil in the pots. These pot cultures were not sealed with wax as is often done for the purpose of eliminating all moisture losses from the soil. To obtain an idea of the amount thus lost, two crocks of soil without plants were run along with the others as a check. When the other cultures received a gravel mulch, these two also received the same. A number of experiments showed that the loss of water by evaporation from the soil was very small and the water loss from the cultures may be assigned to water loss from the plants. LEATHERS in India (6) and others have also found that the water loss from soil was very little and could be neglected from calculation, or determined from control pots and the percentages calculated.

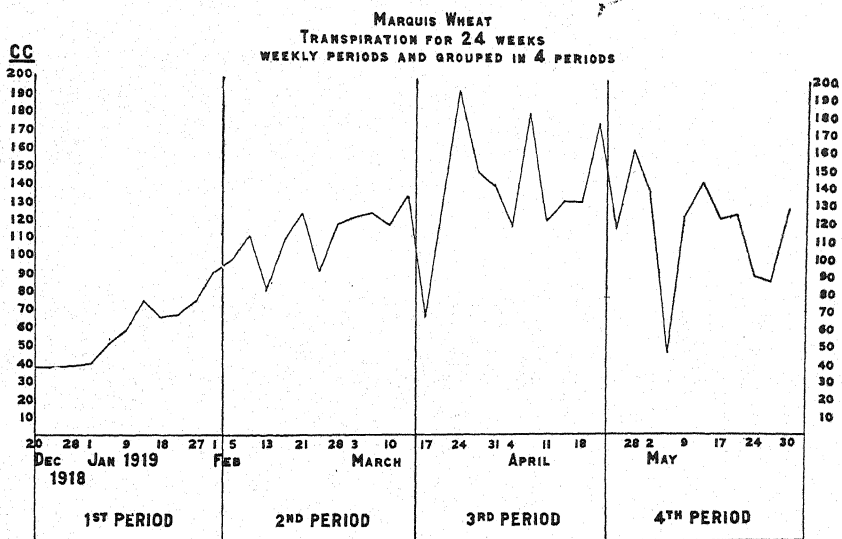


FIG. 7. Mean daily transpiration of all plants throughout the period of growth, plotted by weeks, and divided into four periods.

Transpiration was determined by weight which is more accurate than volume determinations. The first weighings were made on December 20th and every 3.5 days thereafter until June 3rd. Figure 7 is a curve showing the loss of water from soil and plants for the period of December 20 to May 30. The entire period of 161 days has been divided into four smaller periods of five to six weeks duration for the purposes of comparison. It will be observed that the loss at the start is small and this increases gradually until it reaches a maximum at the latter part of March, just a short time before the heading out of the grain, and then it falls off to the last day. The curve resembles a growth curve in that there is a grand period of water loss like the "grand period of growth." During the heading out period, the greatest amount of water is called for, and transpiration is greatest at this time. It suggests that transpiration may be a good measure of plant growth. Curves for mean daily transpiration and evaporation ratio for two-week periods are shown in figure 8. The transpiration data are not given.

WATER REQUIREMENT

The amount of water loss for each culture for every 3.5 day period was determined, but the data are not given here. Since the water requirement

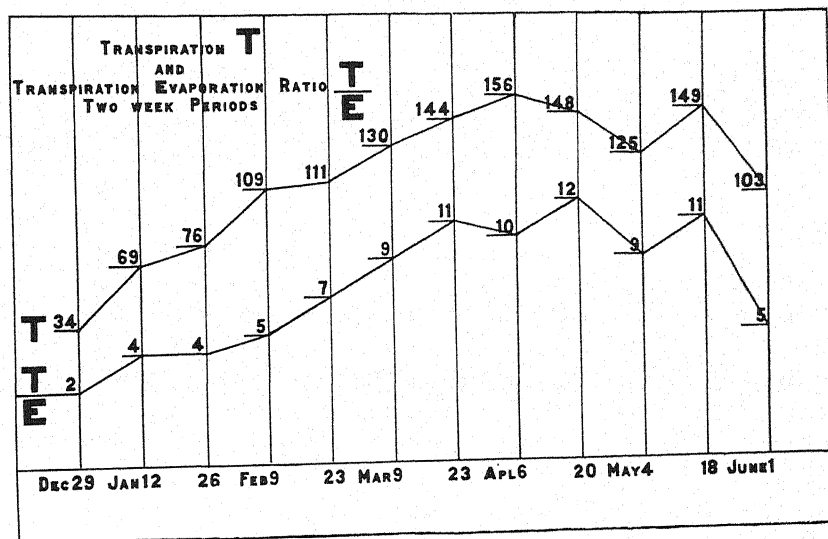


FIG. 8. Mean daily transpiration T , in cc. for two-week periods. Mean daily transpiration- $\frac{T}{E}$, calculated from cc. in two-week periods.

is the ratio between the amounts of water lost by transpiration and the dry weight yield, it may be used as a criterion of growth. The interesting feature of the water requirement data is the evidence that the high yielding cultures have a lower water requirement than any of the other cultures (1, 7, 2; 1, 8, 1; 2, 5, 3; 4, 1, 5; 4, 2, 4; 4, 3, 3; 5, 3, 2; 6, 2, 2; and 8, 1, 1). The highest yielding nine cultures have a water requirement of approximately 600 or less, while the remaining cultures are above this. The best salt proportions of the fertilizer ingredients apparently give a low water requirement, and the poorer salt proportions give the highest. Not only is it true that increasing amounts of the fertilizer mixture, up to a certain limit, lowers the water requirement, but also a change to the proper ratio of the fertilizer ingredient seems to decrease the water requirement.

The experiment was so planned that the data could be subjected to statistical treatment. There were twelve plants in each of the two control pots, providing data on 24 plants to be used for comparison with the 12 plants in each of the other cultures. Student's method and BESSEL'S were used in making these comparisons and significant differences were found, so that the highest yielding cultures fell in one group and the lowest yielding cultures fell in another group just as can be shown by the actual results of yields, etc.

However, this sort of statistical treatment is not necessary in the present work where an explanation of the scattering of the good cultures over the triangular diagram is under consideration. Consequently a discussion of these results is omitted.

Field experiment

The experiment reported in this section was our first attempt at applying a wide range of salt proportions in the field. The three fertilizers commonly used in a complete fertilizer mixture, calcium acid phosphate, sodium nitrate, and potassium sulphate, were the compounds or salts used. The soil was taken just as it was without a study of its chemical, physical and biological characteristics, and treated with the various proportions or combinations of fertilizer salts. The influence of the fertilizer is judged according to the effect it has on the growth of oats taken as the indicator. This same general plan has been followed for three successive years following the date of this experiment.

It was expected that many of the details of the method employed would have to be changed or modified as experience dictated, and that some methods would have to be discarded. The experiment was merely a preliminary one and many suggestions for guidance in future work have come out of it.

There was no special preparation of this small strip of level land, approximately 500 feet long and 60 feet wide, which was set aside for this experiment. The crop of the preceding year was beans. Early in the following spring, manure was spread on, and plowed under shortly afterwards. It was plowed in the usual way and then the seed bed was prepared for the drilling of oats. Worthy oats obtained from the Farm Crops Department were first treated with formaldehyde for smut and then planted at the rate of six pecks to the acre. Planting was done on May 8th, the rows running the long way of the field. The weather was good and the stand excellent.

A couple of days before the fertilizer was applied, the ground was staked out. The plan of series C is shown in fig. 9; series B and A, not shown in the figure, are continuous towards the east. Each series is separated from its neighbor by an alley five feet wide running across the rows. There were forty-four plots in each series, each plot being forty feet long and two feet wide. Each plot is made up of four rows of plants, the two center ones being taken as the experimental rows. The fertilizer was distributed between these on June 5th. At this time the plants were six to eight inches tall. Several border rows bounded the strip of land on sides and ends. No check or fertilizer plot was placed on the dead furrow. Before harvesting, the two outer rows in each plot were pulled up and this aided considerably in marking off clearly the fertilized and check rows so that harvesting could be quickly done. The check and fertilized rows were hand cut August 9th, and the rest machine cut at a later date.

N

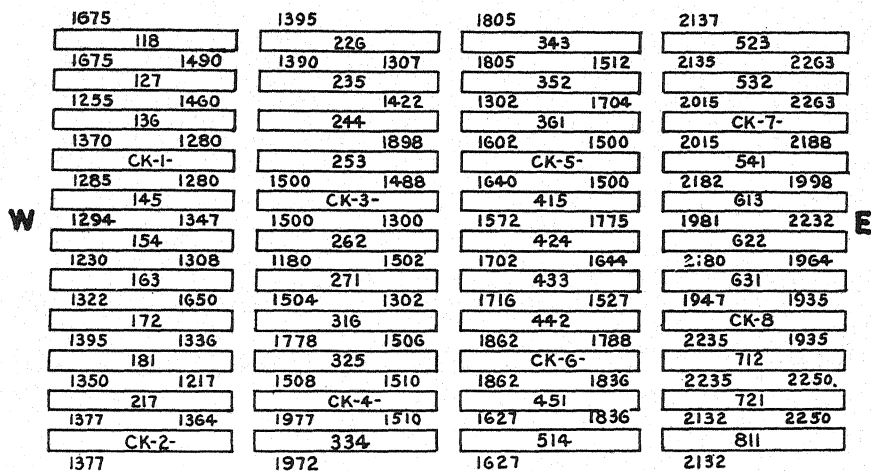


FIG. 9. Arrangement of field plots, series C. Numbers in rectangles show proportions of fertilizers. See text for details.

In fig. 9, the numbers in the rectangular blocks represent the proportions of the three salts, distributed on the basis of ten per cent. increments. The first number stands for calcium acid phosphate, the second for sodium nitrate, and the third for potassium sulphate. The fertilizer mixture for plot 5, 2, 3 then contains 5 parts of calcium acid phosphate, 2 parts of

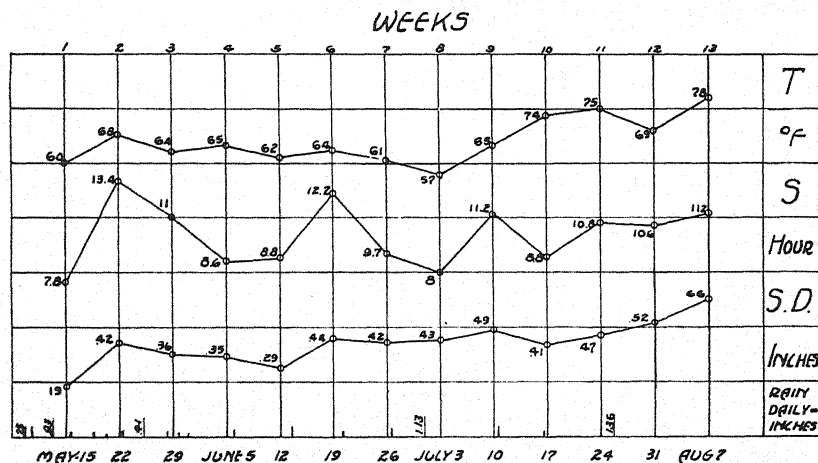


FIG. 10. Mean daily temperature, T, in °F.; mean daily sunshine, S, in hours; mean daily saturation deficit, S.D., in inches; and daily rainfall in inches, for each week of season.

TABLE VII

QUANTITIES OF FERTILIZER, AND ACTIVE INGREDIENTS PER PLOT. TOTAL AMOUNT OF FERTILIZER PER PLOT, 2 POUNDS (1089 POUNDS PER ACRE) IN 10 PER CENT. STAGES

PLOT NUMBER	ACID PHOSPHATE 16.5 PER CENT P_2O_5		SODIUM NITRATE 15.1 PER CENT. N		POTASSIUM SUL- PHATE 47.79 PER CENT. K_2O		TOTAL ACTIVE INGREDIENTS
	Ferti- lizer	Active ingre- dient	Ferti- lizer	Active ingre- dient	Ferti- lizer	Active ingre- dient	P, N, K
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1, 1, 8	91	15	91	14	726	347	376
1, 2, 7	91	15	181	27	635	303	345
1, 3, 6	91	15	272	41	544	260	316
1, 4, 5	91	15	363	55	454	217	287
1, 5, 4	91	15	454	69	363	173	257
1, 6, 3	91	15	544	82	272	129	226
1, 7, 2	91	15	635	96	181	87	198
1, 8, 1	91	15	726	110	91	53	178
2, 1, 7	181	30	91	14	635	303	347
2, 2, 6	181	30	181	27	544	260	317
2, 3, 5	181	30	272	41	454	217	288
2, 4, 4	181	30	363	55	363	173	258
2, 5, 3	181	30	454	69	272	129	228
2, 6, 2	181	30	544	82	181	87	199
2, 7, 1	181	30	635	96	91	53	179
3, 1, 6	272	45	91	14	544	260	319
3, 2, 5	272	45	181	27	454	217	289
3, 3, 4	272	45	272	41	363	173	259
3, 4, 3	272	45	363	55	272	129	229
3, 5, 2	272	45	454	69	181	87	201
3, 6, 1	272	45	544	82	91	53	180
4, 1, 5	363	60	91	14	454	217	291
4, 2, 4	363	60	181	27	363	173	260
4, 3, 3	363	60	272	41	272	129	230
4, 4, 2	363	60	363	55	181	87	202
4, 5, 1	363	60	454	69	91	58	182
5, 1, 4	454	75	91	14	363	173	262
5, 2, 3	454	75	181	27	272	129	231
5, 3, 2	454	75	272	41	181	87	203
5, 4, 1	454	75	363	55	91	53	183
6, 1, 3	544	90	91	14	272	129	233
6, 2, 2	544	90	181	28	181	87	204
6, 3, 1	544	90	272	41	91	53	184
7, 1, 2	635	105	91	14	181	87	206
7, 2, 1	635	105	181	27	91	53	185
8, 1, 1	726	120	91	14	91	53	187

sodium nitrate and 3 parts of potassium sulphate. This makes a total of ten parts of all the ingredients. The proportions for all the other fertilized plots can be determined in like manner. One plot in each of the first third, and fourth rows was set aside for single fertilizer applications.

The application of fertilizer was at the rate of 2 pounds to the plot in all cases. The amount of active ingredient was much less since the fertilizer contained a filler. The amount of fertilizer and active ingredients per pot in grams is found in table VII. It will be noted from this table that the individual ingredients vary progressively, but that the total concentration of fertilizer in each plot is not the same. In the case of water cultures strict attention is given to this feature so that all cultures have the same total concentration. In regard to soil plots in the field, the concentration cannot be controlled, for it differs at different times under the varying climatic and edaphic conditions.

All these various fertilizer combinations were prepared beforehand and put into separate paper sacks, clearly labelled. They were then taken to the field and placed on their respective plots to avoid any chances of mistake. One person spread the entire set of combinations for the three series, this being deemed necessary for uniform distribution. After a little practice these various fertilizers can be uniformly spread. A light rain fell the evening after the fertilizer was spread. Two days later a heavier rain fell and helped considerably to incorporate the fertilizer with the soil. The field was kept fairly free from weeds by hoeing. When the plants became large enough to shade the growth beneath, weeding became necessary. The stand of oats was an excellent one and the climatic conditions were especially conducive to good growth during the season.

ENVIRONMENTAL CONDITIONS

Tables follow showing the temperature, rainfall, saturation deficit, and sunshine data effective during growth. The data from which these tables were made were kindly given us by Mr. D. A. SEELEY, of the local Weather Bureau.

In the form usually given the data do not represent exact conditions, for the means or averages give an inadequate idea of the influence of the various climatic conditions on the plant. Obviously, the intensity and duration of the extremes of the various factors play a larger rôle in the plant's development than if the plant were exposed for a long period at some point between the extremes. This may be illustrated in the following manner: There is a certain stage in the life of the oat plant when water is needed more than at another time. This is the critical period as far as water is concerned. During the first ten days after blossoming the

plant should have an abundance of moisture to help fill out the grain and bring the head to perfection. If moisture is lacking at this time the result is consequently poor grain. In a period like this, abundant moisture is not a detriment, but if the temperature is high and the season dry, deleterious effects are produced. The present season had no extremes that held for long periods. This may be observed by a study of the table.

In table VIII, the mean daily temperature, sunshine, saturation deficit and rainfall are recorded in weekly periods. Temperature is given in degrees F., sunshine in hours, saturation deficit and the rainfall, in inches. The conditions are shown graphically in fig. 10.

TABLE VIII

MEAN DAILY TEMPERATURE T, SUNSHINE S, SATURATION DEFICIT S.D., AND RAINFALL R,
RECORDED IN WEEKLY PERIODS

WEEKLY PERIODS	TEMPERATURE	SUNSHINE	SATURATION DEFICIT	RAINFALL
	°F.	hours	inches	inches
May 8-May 15	60	7.8	0.19	0.20
May 16-May 22	68	13.4	0.42	0.03
May 23-May 29	64	11.0	0.36	0.10
May 30-June 5	65	8.6	0.35	0.01
June 6-June 12	62	8.8	0.29	0.02
June 13-June 19	64	12.2	0.44	0.03
June 20-June 26	61	9.7	0.42	0.03
June 27-July 3	57	8.0	0.43	0.22
July 4-July 10	65	11.2	0.49	Trace
July 11-July 17	74	8.8	0.41	0.08
July 18-July 24	75	10.8	0.47	Trace
July 25-July 31	69	10.6	0.52	0.20
August 1-August 7	78	11.2	0.66	0.01

The saturation deficit for the week is obtained by averaging the daily saturation deficits. The daily saturation deficits are derived from the noon temperatures and the vapor pressures for that day. The vapor pressure is found in the Weather Bureau psychrometric tables. When the observed vapor pressure for any particular day is subtracted from the vapor pressure at saturation for that day the saturation deficit is obtained. It is believed that this gives a better criterion of the moisture condition of the air than relative humidity and for this reason the method has been employed (11, 8).

Discussion of results

As is the usual custom, grain yield is taken here as the criterion in determining the influence of the different salt combinations. When the plants

TABLE IX

ACTUAL YIELDS AND RELATIVE WEIGHTS OF GRAIN OF OATS GROWN IN THE FIELD FROM MAY 8TH TO AUGUST 9TH. EXPERIMENT RUN IN TRIPPLICATE, A, B, C

PLOT NUMBER	YIELD			YIELD			
	A	B	C	RATIO TO CHECK AS UNITY			
				A	B	C	AVERAGE
	gm.	gm.	gm.				
1, 1, 8	1600	1220	1675	1.00	0.83	1.03	0.95
1, 2, 7	1298	1490	0.88	0.92	0.90
1, 3, 6	1325	1310	1255	0.83	0.87	0.77	0.83
1, 4, 5	1527	1372	1285	0.96	0.92	0.79	0.89
1, 5, 4	1262	1255	1347	0.79	0.85	0.83	0.82
1, 6, 3	1235	1325	1230	0.77	0.90	0.76	0.81
1, 7, 2	1343	1212	1650	0.84	0.83	1.01	0.89
1, 8, 1	1200	1635	1395	0.75	1.10	0.86	0.90
2, 1, 7	1410	1590	1217	0.88	1.07	0.75	0.90
2, 2, 6	2050	1395	1.29	0.86	1.07
2, 3, 5	2100	1235	1397	1.32	0.84	0.81	0.99
2, 4, 4	1625	1390	1.02	0.94	0.98
2, 5, 3	1450	1368	1898	0.91	0.92	1.17	1.00
2, 6, 2	1675	1050	1300	1.05	0.71	0.60	0.85
2, 7, 1	1637	1387	1180	1.03	0.94	0.73	0.90
3, 1, 6	1530	1245	1312	0.96	0.78	0.79	0.84
3, 2, 5	1300	1530	1778	0.84	1.03	1.09	0.98
3, 3, 4	1425	1300	1972	0.90	0.89	1.21	1.00
3, 4, 3	1720	1795	1805	1.08	1.21	1.11	1.13
3, 5, 2	1765	1400	1512	1.11	0.94	0.93	0.99
3, 6, 1	1520	1497	1367	0.96	1.01	0.84	0.93
4, 1, 5	1857	1612	1650	1.16	1.09	1.01	1.09
4, 2, 4	1475	2075	1775	0.93	1.39	1.09	1.13
4, 3, 3	1630	1435	1702	1.03	0.97	1.04	1.01
4, 4, 2	1712	1710	1527	1.08	1.15	0.94	1.06
4, 5, 1	1307	1500	1835	0.82	1.01	1.13	0.98
5, 1, 4	1952	1542	1627	1.23	1.02	1.00	1.08
5, 2, 3	1032	1645	2135	0.65	1.11	1.31	1.02
5, 3, 2	1713	1450	2263	1.07	0.98	1.39	1.14
5, 4, 1	1785	1740	2188	1.12	1.17	1.34	1.21
6, 1, 3	2020	1690	2182	1.27	1.14	1.34	1.25
6, 2, 2	1280	1258	2232	0.84	0.85	1.37	1.02
6, 3, 1	1853	1452	2180	1.16	0.98	1.34	1.16
7, 1, 2	1970	1635	2235	1.24	1.10	1.37	1.24
7, 2, 1	1342	1540	2250	0.84	1.02	1.38	1.05
8, 1, 1	1952	1637	2132	1.29	1.10	1.31	1.23

Average of 8 controls Series A, 1589; B, 1477; C, 1622.

on each plot were harvested, they were tied together in a bundle and allowed to dry. Later they were thrashed in a small machine by hand. These grain weights given in the table are not to be considered as absolutely correct since there are losses in the field by shattering, by birds, by transportation and by thrashing. These losses are comparatively small and perhaps uniform with all bundles.

The figures above the rectangles in fig. 9 represent the actual yields in grams, the first one to the left end of the first rectangle and the second to the right end of the second rectangle, and so on, arbitrarily arranged in this way to avoid crowding. The figures under the rectangles represent the normal yields calculated from the two nearest checks on the assumption that the fertility of the soil undergoes a gradual change from one check plot to the next. The yields of the fertilized plots between two checks were thus corrected. The yields of the fertilized plots beyond the checks to the end of the experimental strip were calculated in the same way, using the nearest check and the highest or lowest yielding plot as the case might be, when the fertility of the intervening space increased or decreased.

The actual yields and the relative weights of grain for the three series are given in table IX. In columns 5, 6, and 7 the relative yields are given in ratios, taking the values of the control as unity. In series A, all the checks averaged 1589 grams; in series B 1477 grams; and in series C, 1622 grams. In the last column, the average of all three series is given. The normal yields and relative weight of grain for series A, B, and C are given in table X. The ratios of fertilizer plots to check plots, with check as unity, are also given for the three series. The good and bad yields are represented on the triangular diagram as shown in fig. 11.

A study of the tables and figures reveals the fact that the plots in series C as a whole showed the highest yields. Series A followed next, and then series B. A study of each series showed that as far as high and low yields were concerned there was some similarity. When the data were arranged in the triangular diagram, the high yielding plots were for the most part found in the upper part of the triangle, and the low yielding cultures in the lower part of the triangle. In this respect, the data in Series A and C were more striking than in series B. When an average of the three series was taken, the best yielding cultures were with one exception in the upper angle above the 40 per cent. line. Furthermore, the yields were increasingly heavier as the cultures approach the upper angle. The poorest cultures are for the most part found in the lower part of the triangle in the 10 per cent. line. Consequently, it is apparent that plots high or moderately high in calcium acid phosphate give the highest yields while those low in acid phosphate give the lowest yields. The ill effects of too much K_2SO_4 in the fertilizer is apparent from a consideration of the tables. The cultures

TABLE X

NORMAL YIELD AND RELATIVE WEIGHTS OF OATS IN THE FIELD FROM MAY 8TH TO AUGUST 9TH. EXPERIMENT RUN IN TRIPPLICATE, A, B, C

PLOT NUMBER	YIELD			YIELD			
	A	B	C	RATIO TO CHECK AS UNITY			
				A	B	C	AVERAGE
	gm.	gm.	gm.				
1, 1, 8	1425	1220	1675	0.90	0.83	1.03	0.92
1, 2, 7	1289	1460	0.87	0.90	0.88
1, 3, 6	1325	1288	1370	0.83	0.87	0.84	0.85
1, 4, 5	1547	1288	1294	0.97	0.87	0.80	0.88
1, 5, 4	1794	1287	1308	1.12	0.87	0.81	0.93
1, 6, 3	1839	1386	1322	1.16	0.87	0.81	0.95
1, 7, 2	1636	1361	1336	1.03	0.92	0.82	0.92
1, 8, 1	1433	1437	1350	0.90	0.97	0.83	0.90
2, 1, 7	1410	1590	1364	0.89	1.10	0.84	0.94
2, 2, 6	2050	1400	1390	1.29	0.85	0.86	1.03
2, 3, 5	2100	1380	1422	1.32	0.93	0.88	1.04
2, 4, 4	1719	1360	1466	1.08	0.91	0.90	0.96
2, 5, 3	1528	1340	1488	0.96	0.91	0.92	0.93
2, 6, 2	1377	1320	1502	0.87	0.90	0.93	0.90
2, 7, 1	1417	1387	1504	0.89	0.94	0.93	0.92
3, 1, 6	1457	1348	1506	0.92	0.99	0.93	0.95
3, 2, 5	1497	1530	1508	0.94	1.03	0.93	0.96
3, 3, 4	1538	1300	1672	0.97	0.88	1.03	0.96
3, 4, 3	1720	1795	1805	1.08	1.21	1.11	1.13
3, 5, 2	1650	1673	1704	1.04	1.13	1.05	1.07
3, 6, 1	1600	1756	1602	1.00	1.18	0.99	1.05
4, 1, 5	1560	1839	1572	0.98	1.24	0.97	1.06
4, 2, 4	1569	2065	1644	0.98	1.39	1.01	1.12
4, 3, 3	1579	1888	1716	0.98	1.27	1.05	1.10
4, 4, 2	1588	1710	1788	1.00	1.15	1.10	1.09
4, 5, 1	1598	1500	1835	1.00	1.01	1.13	1.04
5, 1, 4	1952	1542	1627	1.23	1.04	1.00	1.09
5, 2, 3	1363	1645	2135	0.87	1.11	1.32	1.10
5, 3, 2	1713	1450	2263	1.08	0.99	1.39	1.15
5, 4, 1	2025	1503	1998	1.28	1.01	1.23	1.17
6, 1, 3	1987	1580	1981	1.25	1.07	1.22	1.18
6, 6, 2	1949	1603	1964	1.23	1.08	1.20	1.17
6, 3, 1	1911	1626	1947	1.20	1.10	1.17	1.15
7, 1, 2	1873	1635	2235	1.28	1.11	1.37	1.22
7, 2, 1	1874	1600	2250	1.18	1.08	1.38	1.21
8, 1, 1	1952	1637	2132	1.23	1.10	1.32	1.21

Average of eight checks A, 1589; B, 1477; C, 1622.

where high concentrations of this salt are found show lowest yields. The grouping, however, is not ideal except in series C. This is the state of affairs when actual yields are considered.

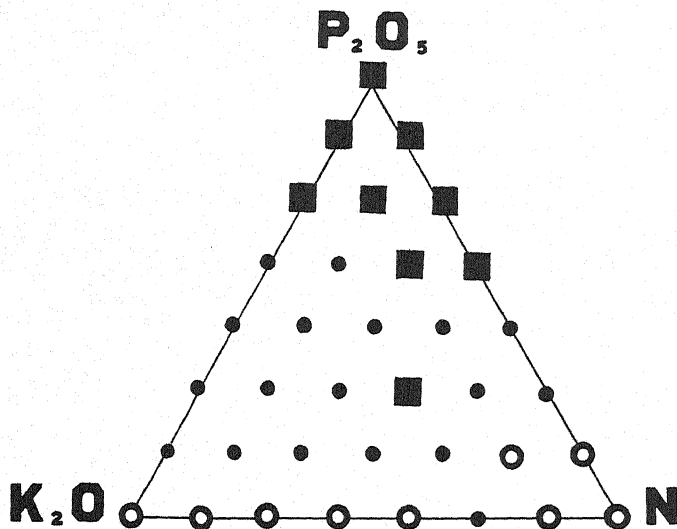


FIG. 11. High and low yielding cultures when *normal* yields are taken. The best cultures, ■ ; the poorest cultures, ○.

When normal yields were considered, better grouping of high yielding cultures was obtained with one exception, plot 3, 4, 3. Low yielding cultures are at the lower side of the triangle where acid phosphate is low in concentration.

For the particular soil and for the climatic and edaphic conditions under which the experiment was performed, the data showed that best growth was obtained where cultures were high in calcium acid phosphate and poor growth followed where low concentrations of calcium phosphate were used. This is in agreement with the general observation that cereals do best with comparatively high applications of acid phosphate.

The result of this experiment conducted in the field gave better proof of the need of a narrow range of ratios than the one conducted in the greenhouse. A single experiment like this, however, does not give one the needed proof that it is generally true, although it may look so. The experiment was not repeated the following year, the land having passed out of our hands. It is not known, therefore, whether the same results could have been obtained with repetition on the same field. Our chief concern was

to determine whether there was any need of a narrow range of ratios on a single soil type, during a single season.

Since the above experiment was completed, three other similar ones were conducted in different fields for the growing season of three successive years, 1919, 1920, and 1921. The high yields were not grouped together, nor were the low yields centered in any particular region. Thus, different types of soils have been tested during the four different seasons in an attempt to discover whether there is a definite grouping in response to salt ratios. Although tendencies indicating grouping were evident at times, one is not justified in concluding that for general practice any definite ratios are required. In fact, a rather wide range of ratios proved equally good. Increasing the application of active ingredients from 100 pounds to 160 pounds per acre, and running the experiment in duplicate did not change the general results. Decreasing the total amount of active ingredient brought no change in the general result. Three acres of corn on a farm near Clio, Michigan, yielded no better results. If one had carried on the experiment in the same field, year after year, the fertility might have been built up and the yields per plot greatly altered, but this is another type of experiment in itself, and one that was not attempted.

A more detailed account of the various experiments mentioned above is unnecessary. The results are of such a nature as to indicate that mineral salt ratios for good development of oats and other crops are not sharply defined in soil cultures. It seems improbable that such experiments will contribute much to the development of a rational fertilizer practice.

Summary

This paper presents the results of an experimental study of the theory of physiological balance in soil cultures. The method of attack is that suggested in 1910 for water culture investigations by SCHREINER and SKINNER and further developed by B. E. LIVINGSTON and his collaborators; in short it is the well known triangular system for determining the best salt ratio or combination for plant growth. The first section of the paper deals with the growth of Marquis wheat in soil in 2-gallon glazed pots. This experiment was conducted in the greenhouse during the period from December 16, 1918, to June 9, 1919. The plants were grown to maturity. The second section deals with the growth of Worthy oats in the field, during the period from May 8, 1918, to August 9, 1918. These plants also were grown to maturity.

In both experiments the soil was treated with all the possible combinations of the three ingredients in the fertilizer mixture when these were made arbitrarily to vary by increments of ten per cent. Consequently

there were thirty-six different ratios or combinations. The salts or fertilizer ingredients for both experiments were taken from the same supply and consisted of calcium acid phosphate, potassium sulphate, and sodium nitrate. The soil for the experiment reported in the first section of this paper was taken from the surface at different places scattered over the field, in which was conducted the experiment described in the second section.

In the greenhouse experiment each pot, and in the field experiment each row, represented a single fertilizer ratio or combination. Besides these ratios of the three salts, there were in both experiments single fertilizer applications for the purpose of comparison. In each experiment there were several control cultures.

The experiment in the field was run in triplicate. Each series, separated by alleys five feet in width, was made up of plots forty feet by two feet. Each plot contained four rows of plants, the two center rows receiving the fertilizer application broadcasted between them, when the plants had reached an average height of six to eight inches. In each series there were eight check rows scattered among the plots.

Certain observations were made on the environmental conditions surrounding the plants in both experiments. The importance of these observations is emphasized. The data collected were subjected to statistical analysis. This field experiment was preliminary in nature and the forerunner of three other series, one for each of the growing seasons of 1919, 1920, and 1921. The main conclusions of this study can be briefly stated.

The data, along with other unpublished data, suggest that nothing can be gained by attempting, through the use of the triangular system of fertilizer ratios, to find a definite ratio of salts, or in other words a physiological balance in soil cultures. The evidence does not indicate that we are justified in drawing any other conclusion than that for cereals, such as wheat, oats, and corn, the yields may be quite identical under somewhat wide variations of fertilizer ratios.

The many factors that were suggested as possible agents in producing the negative results were considered, but no satisfactory explanation has been found. It is suggested that the crops tested have been for generations so modified by domestication that they have become accustomed to wide variations in environmental conditions and for that reason are not suitable indicators for studies of this kind. Results might have been different with some wild variety raised in a restricted environment. It is quite apparent from knowledge already acquired that different proportions of the essential elements are required for the different phases of growth. These proportions probably vary also from week to week through the life of the plant, chiefly because there is a variation in the salt content of all ordinary soils. The

writer is convinced that a rather wide range of salt ratios can be used without causing appreciable differences in yields.

Under the conditions of these experiments the use of single fertilizer ingredients does not give as good results in soil cultures as a combination of three. Other experiments not only in soil cultures but in water cultures agree with and emphasize this conclusion. Only under conditions where the price for a mixture of the three fertilizer ingredients is prohibitive would the use of single fertilizers be advisable.

MICHIGAN STATE COLLEGE,
EAST LANSING, MICHIGAN.

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ON THE PASSAGE OF BORIC ACID AND CERTAIN SALTS INTO FRUITS AND VEGETABLES

LOUIS KAHLENBERG AND RALPH TRAXLER

Introduction

In a previous paper¹ KAHLENBERG showed that boric acid passes through the living human skin by osmosis. This work suggested that boric acid might similarly pass into fruits and vegetables immersed in solutions of the acid. Experiments were not confined to boric acid alone, but a number of salts were tested to see if they would permeate the integuments of the same fruits and vegetables. The salts chosen for this purpose were Li_2SO_4 , LiCl , LiNO_3 , $\text{Na}_2\text{B}_4\text{O}_7$, $\text{Li}_2\text{B}_4\text{O}_7$, KI , BaCl_2 , and SrCl_2 . In a few instances still other salts were employed which will be mentioned in connection with the specific experiments to be described. The fruits and vegetables tested were cherries, strawberries, plums, gooseberries, grapes, tomatoes, cucumbers, carrots, peaches, and apples.

The method of experimentation was quite simple. It consisted of suspending the fruit in a solution of known strength of each of the substances mentioned, and then after a measured time determining whether any of the dissolved substance had entered the fruit. All of the substances used were chemically pure and they were dissolved in carefully distilled water. Usually the solution contained one-tenth of a gram molecule of the substance per liter, though in some cases saturated solutions were employed. The analysis of the fruit at the conclusion of the experiment was generally readily effected. Boric acid and borates could easily be detected by the turmeric test, and the spectroscope was used to detect lithium and strontium. Barium was detected by means of sulphuric acid, and iodine by the starch test. Before making the analysis the fruit was, of course, in each case very carefully washed with distilled water.

All of the fruits used were chosen with great care, and any fruits that showed imperfections were discarded. The experiments were conducted at least in triplicate in each case so as to guard against the possibility of error due to individual peculiarities of the fruit used. Moreover, the fruits employed were perfectly sound and had not been subjected to cold storage treatment except in a few cases where fruits which had been in cold storage were purposely employed to determine what effect such cold storage would have. In every case blank tests were made on the untreated fruit.

Before immersing fruits in the solutions, they were carefully washed

¹ KAHLENBERG, LOUIS, Jour. Biol. Chem. 62: 149-156. 1924.

with distilled water and gently dried with a soft clean cloth. In a few instances the fruits were washed with alcohol, ether, chloroform, benzol, acetone, carbon bisulphide, etc., to remove the outer waxy coat before introducing the fruit into the solution. The results of these special experiments will be given later.

In immersing the various fruits special care was taken not to have either the stem ends or blossom ends dip into the liquids. All experiments were performed at room temperatures, about 20° C.

Results of experiments

CHERRIES.—The experiments with cherries were done with unripe sour cherries, with ripe sour cherries, and with ripe black California cherries. The unripe sour cherries were picked in the orchard of the Agricultural College of the University of Wisconsin at a time when the fruit was just beginning to turn white. The fruit was picked so that two cherries were connected by their stems. Each such pair was then hung over a small stick placed across the top of a crystallizing dish 10 cm. in diameter. Not over 30 minutes elapsed between the time of picking and of placing in the solution. The results are as follows: In 5.5 hours saturated H_3BO_3 solution, 0.1 molar LiCl , 0.1 molar LiNO_3 , and 0.1 molar Li_2SO_4 passed into the cherries in detectable quantities. These amounts were greatly increased in 22 and 30 hours, respectively. A 0.1 molar solution of $\text{Na}_2\text{B}_4\text{O}_7$ passed into the cherries very slightly in 5.5 hours, but after 22 and 30 hours quite notable amounts were present in the fruit. It took 22 hours before 0.1 molar H_3BO_3 was found in the cherries in detectable quantity. Similarly, a saturated solution of $\text{Li}_2\text{B}_4\text{O}_7$ did not enter the fruit in notable amounts until after 22 hours. Even at that time, the tests for lithium and boron in the cherries were but slight. In 0.1 molar Li_2SO_4 they burst after 22 hours' immersion.

Similar experiments made with ripe sour cherries from the same trees yielded the following results: From a saturated solution, boric acid did not pass into the fruit until after it had been immersed for 21 hours. Similarly, it required 22 hours for lithium to make its appearance in the cherries from 0.1 molar solutions of LiCl , LiNO_3 , and Li_2SO_4 . From 0.1 molar H_3BO_3 it required 30 hours before the boric acid could be detected, while from 0.01 molar $\text{Li}_2\text{B}_4\text{O}_7$ but mere traces of lithium and boron were found in them after 53 hours. A 0.1 molar solution of BaCl_2 did not enter in detectable quantities until 52 hours had elapsed, while 0.1 molar SrCl_2 did not enter at all.

The leaves adjacent to the growing cherries were immersed in each of the solutions above mentioned in order to ascertain whether any of the dissolved

substances would thus pass into the fruit. These experiments were run from 5 to 70 hours, but in no case was even a trace of boric acid or the various salts found in the cherries.

California cherries were treated in the same manner as the sour cherries. The identical solutions already mentioned were used throughout. In addition, however, 0.1 molar solutions of each of the following were also employed: Sodium acetate, CdBr_2 , NaF , KCl , NaCl , urea, Na_2SO_4 , K_2SO_4 , HCl , HNO_3 , acetic acid, and distilled water. In most of these cases the cherries burst, the only exceptions being in the solutions of BaCl_2 , SrCl_2 , CdBr_2 , and saturated solution of Na_2SO_4 . In these latter cases no effect whatever was noted, though the experiments with BaCl_2 and SrCl_2 were continued for 54 hours and that with CdBr_2 for 150 hours. The saturated solution of Na_2SO_4 was used for 10 hours. All experiments were run at least in triplicate and continued till the cherries burst or clearly showed signs of decay. The bursting occurred at somewhat different times in the various solutions but generally in from 4 to 16 hours. The cherries were tested before they had actually burst, but in no case had any of the substances entered into them in detectable amounts before the bursting occurred.

The California cherries were bought at a fruit store in Madison. They had been shipped in refrigerator cars. The fruit was dark red, firm, and apparently in excellent condition. Immersed in distilled water, these cherries burst in from 4 to 5 hours. In the various solutions, the bursting did not occur until the fruit had been immersed somewhat longer, except that in 0.1 molar Na_2SO_4 the bursting occurred in 2 hours and in 0.05 molar H_2SO_4 , HCl , and HNO_3 the bursting occurred in about 15 minutes.

STRAWBERRIES.—Fresh ripe strawberries were obtained from the beds of the Agricultural College. Due to unfavorable weather, the crop was small and for this reason but few experiments were made. Berries that had been shipped in were found to be unsuitable because they were all somewhat bruised.

Tiny hammocks of cheesecloth were suspended in the solutions, and the strawberries were placed in these hammocks in such a manner that the fruit was about half immersed in the solutions. The solutions consisted of saturated H_3BO_3 , saturated $\text{Li}_2\text{B}_4\text{O}_7$, and 0.1 molar $\text{Na}_2\text{B}_4\text{O}_7$, H_3BO_3 , LiNO_3 , Li_2SO_4 , and LiCl . In each of these cases the substances had passed into the strawberries in detectable amounts at the end of 5 hours. It was observed that the Li_2SO_4 passed into the strawberries in copious quantities as compared with the other substances used.

PLUMS.—The plums used in the experiments were: California blue "Diamond" plums, California yellow plums, and red "Miner" plums grown near Madison. The California plums were purchased at a local fruit store. They were in excellent condition and had probably been exposed to refrig-

eration in shipment. The blue plums were large, measuring 4 or 5 cm. in diameter, and the yellow ones were of about the same size. The blue and red plums were immersed in the following solutions, all experiments being done in triplicate at least: Saturated H_3BO_3 , saturated $\text{Li}_2\text{B}_4\text{O}_7$, and 0.1 molar H_3BO_3 , LiCl , Li_2SO_4 , LiNO_3 , $\text{Na}_2\text{B}_4\text{O}_7$, SrCl_2 , and KI . The plums burst in 0.1 molar H_3BO_3 after 72 hours, in 0.1 molar LiNO_3 in 46 hours and 0.1 molar KCl in 44 hours. In saturated H_3BO_3 solution some of them burst in 36 hours. Li_2SO_4 penetrated the fruit more rapidly than any other salt. The other substances followed in the following order: LiNO_3 , LiCl , $\text{Li}_2\text{B}_4\text{O}_7$, saturated H_3BO_3 , $\text{Na}_2\text{B}_4\text{O}_7$, KCl , BaCl_2 , SrCl_2 . In fact only after 46 hours was it possible to detect H_3BO_3 in the plums from the saturated solution. No H_3BO_3 passed into the plums from the 0.1 molar solution, this experiment coming to an end in 72 hours when the plums burst. The $\text{Na}_2\text{B}_4\text{O}_7$ did not enter until 72 hours had elapsed. From the $\text{Li}_2\text{B}_4\text{O}_7$ solution some of the salt had passed into the fruit at the end of 27 hours, it being possible to get a spectroscopic test for lithium, but not a turmeric test for boron, which is not surprising since the latter test is less sensitive.

In no case did SrCl_2 , BaCl_2 or KCl enter. In SrCl_2 the plums were immersed for 72 hours; in BaCl_2 for 96 hours; and in KCl for 44 hours, when the fruit burst.

Only a limited supply of yellow plums could be obtained, hence only a few experiments could be conducted with them. Here again, it was observed that neither SrCl_2 nor BaCl_2 entered the plums, though the experiments were run for 77 hours. After immersion in saturated H_3BO_3 solution for 48 hours, the boric acid had entered. In 0.1 molar H_3BO_3 the plums burst badly in 36 hours. The same occurred in 48 hours in 0.01 molar Li_2SO_4 and LiNO_3 entered the plums in notable amounts after 48 hours, while during that time $\text{Na}_2\text{B}_4\text{O}_7$ had entered in but mere traces.

The red plums had just been picked and had not been exposed to refrigeration. They were from 2 to 2.5 cm. in diameter, had red skins and a deep yellow pulp. The experiments clearly showed that these plums were much more permeable to the substances used than the yellow and blue California plums.

Immersed in 0.1 molar H_3BO_3 these plums burst in every case before any of the acid could be detected in them. The SrCl_2 again did not enter the plums even after 130 hours. A few of them burst in this solution at the end of 7 and 26 hours, though most of them did not burst. BaCl_2 was found in traces after 72 hours and in notable amounts after 144 hours. KI entered the fruit in notable amounts in 22 hours. Li_2SO_4 passed into the plums rapidly, notable quantities being present in 22 hours. However, in this solution some of them burst very badly, even at the end of 3 hours. From 0.1 molar solution H_3BO_3 did not enter. In one case the fruit burst

in 22 hours. A 0.1 molar solution of $\text{Na}_2\text{B}_4\text{O}_7$ entered the plums in notable quantities in 45 hours, but only an extremely faint test was obtained at the end of 22 hours. Saturated $\text{Li}_2\text{B}_4\text{O}_7$ entered in 22 hours in clearly detectable quantities. One of the plums burst in the solution at the end of that time. A 0.1 molar LiCl solution passed into the plum in 8 hours, although the test was quite faint; but in 22 hours notable amounts were found in them. Some of the fruit had burst at the end of that time. The LiNO_3 entered the plums a little more rapidly than the LiCl .

Experiments were made with the red Miner plums to see whether the fruit changed in weight while immersed in the solutions already mentioned. These experiments were commonly conducted for 4 hours, though in a few cases they were continued for 72 hours. As a rule the plums showed, if anything, a few hundredths of a gram diminution in weight; but the changes were so slight that they were almost within the limits of experimental error. It is consequently deemed unnecessary to recount all the weighings in tabular form here.

GOOSEBERRIES.—The gooseberries were large perfect fruits freshly picked from the garden. Not more than 30 minutes elapsed between the time of picking and their immersion in the solution. Here again neither SrCl_2 nor BaCl_2 entered the fruit though the experiments were conducted for 192 hours. KCl also found great difficulty in making its way into these berries, mere traces being found to have entered after 120 hours. $\text{Na}_2\text{B}_4\text{O}_7$ also did not pass into the berries for 144 hours, but at the end of 168 hours traces made their appearance in the fruit. H_3BO_3 did not enter from the saturated solution until after 120 hours. From 0.1 molar H_3BO_3 the acid entered in traces after 168 hours, but from 0.01 molar H_3BO_3 none of the acid entered the fruit. On the other hand, from 0.01 molar $\text{Li}_2\text{B}_4\text{O}_7$ lithium entered the berries in 96 hours and both boron and lithium were plainly detectable after 144 hours. 0.1 molar LiCl readily entered the fruit in 25 hours. One of the berries burst in this solution in 12 hours. The LiNO_3 did not make its appearance in the berries until after 48 hours. On the other hand, Li_2SO_4 entered in notable quantities in 48 hours. In this case Li_2SO_4 passed into the fruit more slowly than LiCl .

GRAPES.—Red California grapes, and red, white, and blue Wisconsin grapes were used in these experiments. The California grapes which had been shipped to Madison in refrigerator cars were purchased locally. Each grape was 1.5 cm. in diameter. The Wisconsin red and white grapes were obtained from the vineyards of the Agricultural College. The blue Wisconsin grapes were the so-called "Canton" grapes. They were grown in Madison. In each case from 1 to 2 cm. of the stem was left on the grape to be tested. By means of a thread attached to these stems the grapes were suspended in the various solutions.

In the case of the California grapes neither 0.1 molar SrCl_2 , 0.1 molar BaCl_2 , nor 0.1 molar H_3BO_3 entered the grapes, though the experiments were conducted for 72 hours, and in the case of the SrCl_2 for 96 hours. In the H_3BO_3 the experiments were continued for 144 hours. In a saturated H_3BO_3 solution it took 30 hours for H_3BO_3 to show its presence in detectable quantity in the fruit. The amount increased steadily up to 66 hours, when the experiment was discontinued. From 0.1 molar $\text{Na}_2\text{B}_4\text{O}_7$ the salt passed into them in detectable amount in 48 hours. Very material quantities of $\text{Na}_2\text{B}_4\text{O}_7$ were found in 144 hours. Immersed in saturated solution of $\text{Li}_2\text{B}_4\text{O}_7$, the grapes showed a faint spectroscopic test for lithium in 96 hours. This amount increased slightly up to 156 hours when the experiment was discontinued. However, even at this time not enough of the $\text{Li}_2\text{B}_4\text{O}_7$ had entered to yield the turmeric test for boron. It took 48 hours for lithium to pass into the grapes from 0.1 molar Li_2SO_4 solution. Curiously, even at the end of 96 hours the amount of lithium which had passed into the grape was exceedingly small, being only such traces as could be detected by the spectroscope. From 0.1 molar LiNO_3 , lithium passed into the grapes in detectable amounts in 18 hours, and the quantity increased slightly but steadily up to 68 hours, the duration of the experiment. The grapes immersed in 0.1 molar LiCl exhibited spectroscopic traces of lithium after 24 hours, and the amounts increased notably in 66 hours. The KI entered the grapes from 0.1 molar solution in 18 hours, and the amount continued to increase up to 30 hours when the experiment was stopped.

Occasionally one of the grapes burst during the experiments, but these instances were so few and occurred so irregularly that they were no doubt due to individual differences.

Neither H_3BO_3 , $\text{Na}_2\text{B}_4\text{O}_7$, $\text{Li}_2\text{B}_4\text{O}_7$, KI, SrCl_2 , nor BaCl_2 entered the Wisconsin red grapes, although the experiments were prolonged to 196 hours and in some cases to 264 hours. From 0.1 molar Li_2SO_4 lithium was found in the grapes in 48 hours, and from 0.1 molar LiCl in 72 hours.

The white Wisconsin grapes were the Martha variety. They were found to be impermeable to all the solutions above mentioned, although the experiments were conducted for 120 hours and in some cases even over 300 hours. In fact the experiments were run up to the time when the grapes began to show decay.

Unripe grapes of the same Martha variety were similarly tested. The grapes were picked before any of the fruit on the vines had started to ripen. It was found that these unripe grapes were more permeable than the ripe ones. To be sure, in this case too, neither 0.1 molar BaCl_2 nor SrCl_2 , $\text{Na}_2\text{B}_4\text{O}_7$, H_3BO_3 nor KI entered the grapes even after immersion for 144 hours. The boric acid also did not pass into the grapes from saturated solution in 144 hours. In some cases the experiments were conducted for

268 hours when the grapes began to show signs of deterioration. On the other hand, lithium entered these unripe grapes from 0.1 molar Li_2SO_4 and LiCl in 74 hours; from 0.1 molar LiNO_3 , it passed into them in detectable quantity in 34 hours; and from 0.01 molar $\text{Li}_2\text{B}_4\text{O}_7$ in 144 hours.

The blue Wisconsin grapes known as the Canton grape were rather small, averaging about 1 cm. in diameter. They were carefully picked and at once tested in the laboratory. It was found that neither H_3BO_3 , $\text{Na}_2\text{B}_4\text{O}_7$, LiCl , Li_2SO_4 nor KI entered the grapes, though the experiments were prolonged up to 240 hours.

The same Canton grapes were exposed to refrigeration, being kept in the ice compartment of a refrigerator for 76 hours. The fruit was placed in a box lined with cotton and did not come into direct contact with the ice. It was not frozen, although it had been chilled for 76 hours. The results of the osmotic experiments with these grapes were precisely the same as those with the grapes that had not been chilled.

Some of these Canton grapes were exposed to ultra-violet light from an Hanovia lamp for 2 hours, the grapes being placed about 16 inches from the lamp. The osmotic results with fruit which had been thus irradiated were the same as those with the untreated grapes.

TOMATOES.—Ripe tomatoes grown near Madison were carefully picked and at once immersed in the various solutions. After 6 hours, H_3BO_3 was found in the tomatoes which dipped into the saturated solution. It required 96 hours to get a faint turmeric test for H_3BO_3 in the tomatoes immersed in 0.1 molar H_3BO_3 solution; but in 72 hours 0.1 molar $\text{Na}_2\text{B}_4\text{O}_7$ entered them in detectable quantities. Lithium entered from saturated $\text{Li}_2\text{B}_4\text{O}_7$ solution in 36 hours, from 0.1 molar LiCl in 12 hours, from 0.1 molar Li_2SO_4 in 36 hours, and from 0.1 molar LiNO_3 in 6 hours. From 0.1 molar KI the salt entered in 6 hours. Barium was found in the tomatoes immersed in 0.1 molar BaCl_2 solution in 144 hours, but not a trace of strontium from 0.1 molar SrCl_2 even after 144 hours. Green tomatoes of the same variety were used. They varied from 6 to 8 cm. in diameter. Here again it was found that the green fruit offered less resistance to the solution than did the ripe fruit. In 6 hours H_3BO_3 passed into them from a saturated solution, though it required 36 hours to obtain even a faint test for boric acid in tomatoes immersed in 0.1 molar solution. A 0.1 molar solution of $\text{Na}_2\text{B}_4\text{O}_7$ entered the tomatoes in 36 hours. Saturated $\text{Li}_2\text{B}_4\text{O}_7$ penetrated them in 36 hours, in traces. Lithium passed into these green tomatoes from 0.1 molar Li_2SO_4 in 48 hours, from 0.1 molar LiNO_3 in 36 hours, from 0.1 molar LiCl in 36 hours. The 0.1 molar KI entered in 6 hours but neither BaCl_2 nor SrCl_2 passed into the green tomatoes in 120 hours, the duration of the experiment.

CUCUMBERS.—White spine cucumbers 7 to 10 cm. long and freshly picked were used in these experiments. All of the salts above mentioned entered

these cucumbers in notable quantities in from 16 to 20 hours except SrCl_2 which required 48 hours to make its appearance.

CARROTS.—The carrots, obtained from the College of Agriculture, were grown in soft, loose soil from which they were carefully removed without undue laceration of the rootlets. All of the substances mentioned entered the carrots in from 4 to 10 hours, except 0.1 molar H_3BO_3 which required 23 hours, 0.1 molar SrCl_2 36 hours, and 0.1 molar BaCl_2 24 hours. It was, of course, probable that the substances permeated the carrots where the rootlets were lacerated, it being quite impossible to remove the carrots from the soil without injuring the rootlets. Experiments were consequently performed later by watering the carrots with the various solutions while the plants were left growing in the soil. These results will be reported in another paper. Suffice it here to state that BaCl_2 , SrCl_2 , and KI did not enter the carrots in these experiments, and similar selective qualities were clearly exhibited toward the other substances.

PEACHES.—Perfect Elberta peaches purchased locally were used in these experiments. From saturated H_3BO_3 none of the solute entered the peaches till after 12 hours. It required 24 hours for H_3BO_3 to make its appearance in the peaches from 0.1 molar H_3BO_3 , and 0.1 molar $\text{Na}_2\text{B}_4\text{O}_7$ entered in 44 hours, saturated $\text{Li}_2\text{B}_4\text{O}_7$ in 33 hours, 0.1 molar LiCl in 9 hours, 0.1 molar Li_2SO_4 in 24 hours, 0.1 molar LiNO_3 in 6 hours, 0.1 molar BaCl_2 in 24 hours, 0.1 molar KI in 12 hours. Again the SrCl_2 did not enter even after 120 hours.

It should be noted here that in some of the solutions in which the peaches were immersed, notably in the H_3BO_3 solutions, brown spots developed on the peaches which extended into the tissue of the fruit. These areas were soft and decayed, and contained H_3BO_3 ; but no H_3BO_3 was found in the clear area around the brown spots. Brown spots also appeared on the peaches immersed in $\text{Li}_2\text{B}_4\text{O}_7$, but LiCl and LiNO_3 caused no such spots to form, though these salts entered the fruit rapidly. On the other hand, Li_2SO_4 caused the skin of the peaches to decay badly. BaCl_2 caused brown spots to appear, and fair amounts of the salts were found in the uninjured part of the peach. KI solution did not discolor the peach skins, although iodine permeated them. In $\text{Na}_2\text{B}_4\text{O}_7$ solutions no brown spots were developed, though the solution became yellow after the peaches had been immersed in it for 40 hours. Finally in the SrCl_2 , the peaches remained unchanged and this salt did not pass in even after 5 days, thus clearly demonstrating the highly selective action exhibited by the fruit.

CRABAPPLE.—The fruit used in these experiments was the Milton jelly crabapple. The apples were small, averaging 3 to 4 cm. in diameter. Salt did not pass into the crabapples from 0.1 molar solutions of BaCl_2 , SrCl_2 , or KI in 192 hours. Boric acid from saturated solution entered the fruit in

96 hours, and the apples immersed in 0.1 molar $\text{Na}_2\text{B}_4\text{O}_7$ solution showed detectable quantities of boron in 120 hours. Lithium from saturated solution of $\text{Li}_2\text{B}_4\text{O}_7$ was detected in the fruit in 48 hours. The 0.1 molar Li_2SO_4 entered the crabapples in 24 hours, 0.1 molar LiNO_3 in 24 hours, and 0.1 molar LiCl in 48 hours.

Further experiments were performed with the Milton jelly crabapples using the solutions above mentioned, but allowing the blossom end of the fruit to come into contact with the various solutions. Here H_3BO_3 from saturated solution entered the apples in 102 hours, from 0.1 molar solution in small amounts in 174 hours, and from 0.01 molar solution not at all in 174 hours, the duration of the experiment. Lithium entered from saturated $\text{Li}_2\text{B}_4\text{O}_7$ solution in spectroscopic amounts in 78 hours. The fruit was permeable to 0.1 molar $\text{Na}_2\text{B}_4\text{O}_7$ in 78 hours, 0.1 molar LiNO_3 in 28 hours; and to 0.1 molar Li_2SO_4 in 28 hours. In 0.1 molar concentration BaCl_2 , SrCl_2 and KI did not pass into the fruit in 192 hours, the duration of the experiments. Very little difference in the rate of entrance of most salts was found in the case of the crabapples when only the sides dipped into the solutions as compared with the tests when the blossom end was immersed in the liquid. In a few cases the substances passed into the blossom end more rapidly than through the sides, showing that in experiments such as these it is best to prevent the solutions from coming into contact with any portion of the skin except the smooth and unbroken side of the fruit.

APPLES.—Wealthy, Talent, Ben Davis, Black Ben Davis and Baldwin apples were used in these experiments. They were all picked in the orchard of the Agricultural College except the Baldwins, which were shipped from Michigan, but had never been in cold storage. In every case, care was taken that the solution did not come in contact with either the stem or blossom end of the fruit. Occasionally an apple was found that developed a decayed spot within a few hours after immersion in the liquid. Apples showing this effect always contained detectable quantities of the substance in the solution. The only explanation of this behavior is that the skins of these particular apples contained minute abrasions, probably caused by handling or shipping, which escaped detection when the fruit was sorted and selected for experimentation. The decayed areas indicated the spot through which the solution found an easy entrance into the apple.

The Wealthy apples were used for experimentation soon after picking from the trees. The fruit averaged 5 to 6 cm. in diameter. Boric acid from saturated solution, and from 0.1 molar solution did not appear in the Wealthy apples even after 400 hours of immersion. Potassium iodide from 0.1 molar solution did not enter in detectable quantities in 240 hours, the duration of the experiment. Although the apples were left in contact with saturated $\text{Li}_2\text{B}_4\text{O}_7$ solution for 240 hours no spectroscopic test for lithium

was found. Sodium baborate from 0.1 molar solution did not enter the apples even after 288 hours, neither did 0.1 molar BaCl_2 , nor SrCl_2 enter after 400 hours. But 0.1 molar LiCl entered in 130 hours, 0.1 molar LiNO_3 in 252 hours, and 0.1 molar Li_2SO_4 in 252 hours.

By peeling one side of Wealthy apples and placing the peeled side in the solution it was found that 0.1 molar BaCl_2 passes through the tissue of the apple very readily and copiously, and the 0.1 molar SrCl_2 appreciably, although these substances do not pass through the apple skins in 400 hours.

Wealthy apples were quickly washed with ether, then with benzene, followed by ethyl alcohol. This treatment removed most if not all of the wax from the skins of the apples, but the washed apples turned brown within a few hours. It was found that benzene was the solvent which caused the greatest injury to the fruit. The washed apples were placed in the above mentioned solutions, and all of the substances entered in notable amounts in 26 hours except the BaCl_2 , which required 96 hours, and the SrCl_2 which did not enter in 150 hours. This behavior of the SrCl_2 showed that the skin was not destroyed, nor were gross openings made during the washings, although the permeability to H_3BO_3 and various salts had been greatly increased.

The action of the vapors of various organic solvents on the skins of Wealthy apples was determined. A pipe stem triangle bent so as to form a tripod was placed in a large beaker, and the apple was put upon this tripod. The liquid to be tested was then carefully poured down the side of the beaker till the bottom of the latter was covered about 1 cm. deep. The beaker was then covered with a large convex glass and allowed to stand at room temperature, about 20°C . The experiment with each apple was usually continued for 24 hours. Benzene, chloroform, ether, carbon tetrachloride, carbon bisulphide, toluene, and acetone were used, each separately, as described. Acetone vapors were the only ones which did not cause marked visible injury to the apple skins. The vapors of the other six solvents caused the skins to be discolored and disintegrated so that when the apples were subsequently immersed in the aqueous solutions the latter readily penetrated the fruit. In an experiment to be mentioned later, apples were treated with boiling hot organic vapors in which case the effect just mentioned was greatly increased.

Wealthy apples were exposed to ultra-violet light from an Hanovia lamp for 2.5 hours. The temperature did not rise above 30°C . During the operation of the lamp a strong odor of ozone could be detected. During and after this treatment the natural odor of the apples was much enhanced.

These apples were found to be more permeable to 0.1 molar $\text{Na}_2\text{B}_4\text{O}_7$, saturated $\text{Li}_2\text{B}_4\text{O}_7$, 0.1 molar LiCl , LiNO_3 and Li_2SO_4 than were the untreated apples. The time required for $\text{Na}_2\text{B}_4\text{O}_7$ to enter decreased from 288

for the untreated, to 216 hours for the irradiated apples, for LiCl from 95 to 56 hours, for LiNO_3 from 250 to 44 hours, and for Li_2SO_4 from 252 to 90 hours. The irradiation did not affect the permeability of the skins toward H_3BO_3 , KI , or BaCl_2 , which fact seems specially noteworthy.

The Talent apples, 7 to 8 cm. in diameter, have skins which are green, with a russet coating in many instances. The substances used passed into the Talent apples much more copiously than into the Wealthy apples. Even 0.1 molar BaCl_2 entered the Talent apples in small amounts in 115 hours. It was found that the Talent apples with russet skins were much more readily permeated than those from the same trees but with clear green skins. Notable amounts of KI and H_3BO_3 were found in russeted apples in 90 hours, whereas no KI or H_3BO_3 was found in the clear green apples until after 115 hours and then only in small amounts. Of the lithium salts, 0.1 molar LiNO_3 entered the clear green apples in 19 hours, 0.1 molar LiCl in 30 hours, and 0.1 molar Li_2SO_4 in 43 hours. The 0.1 molar $\text{Na}_2\text{B}_4\text{O}_7$ entered russet apples in 115 hours, but not the clear skinned apples in 165 hours. The cause of russetting does not seem to be known. It is known, however, that russet apples lose moisture more readily than apples of the same variety without russet.

Talent apples were irradiated with ultra-violet light from the Hanovia lamp used in the previous experiments. In this particular case, however, very little ozone was produced in the neighborhood of the lamp. The irradiated apples showed no increased permeability to any of the solutions used, except in the case of 0.1 molar $\text{Na}_2\text{B}_4\text{O}_7$, which appeared to enter a little more rapidly and copiously than into apples not irradiated.

The Ben Davis apples used were obtained from the orchard of the Agricultural College. The fruit averaged 5 to 6 cm. in diameter. The skins of the Ben Davis apples were more permeable than those of the Wealthy apples and less permeable than the Talent apples to H_3BO_3 and KI . The H_3BO_3 from saturated solution entered in 168 hours and the KI from 0.1 molar solution in 288 hours. Lithium from 0.1 molar LiCl entered the Ben Davis apples in 130 hours. More experiments were not performed because of the small quantity of fruit available.

The Black Ben Davis apples were also 5 to 6 cm. in diameter and were exceptionally firm, which eliminated much of the trouble usually occasioned by bruising. Boric acid from saturated solution entered the Black Ben Davis apples in 140 hours, and 0.1 molar $\text{Na}_2\text{B}_4\text{O}_7$ passed into the fruit in 180 hours. Lithium entered the apples from 0.1 molar LiCl in 24 hours, from 0.1 molar LiNO_3 in 24 hours, and from 0.1 molar Li_2SO_4 in 18 hours. The apples immersed in saturated $\text{Li}_2\text{B}_4\text{O}_7$ contained spectroscopic amounts of lithium in 22 hours. Neither 0.1 molar KI nor BaCl_2 passed into the fruit in 300 hours, while no SrCl_2 could be detected in the tissues of the apples even after 55 hours of immersion.

Lithium malate was prepared from pure Li_2CO_3 and pure malic acid, and Black Ben Davis apples were immersed in a 0.1 molar solution of this salt. Lithium was detected in the apples after 19 hours had elapsed. The salt had passed into the fruit in copious amounts after 22 hours. The lithium thus entered the apples from the lithium malate slightly more rapidly than from LiCl and LiNO_3 and about as readily as from Li_2SO_4 .

To test the influence of high temperatures on permeability, Black Ben Davis apples were exposed to a temperature of 75°C . for one hour in a copper oven with water jacketed walls. Thirty apples weighing approximately 3 kilograms were placed in the oven. While the apples were being heated the wax on the skins became soft and felt greasy when touched with the fingers, and the characteristic odor of baking apples was noticed. After an hour's heating the apples were allowed to come to room temperature, after which they were placed in the various solutions. The color of the fruit was changed from a beautiful red to a reddish-brown. Temperatures higher than 75°C . were tried, but found to cause ruptures in the skins of the apples. The permeability of the skins of these heated apples to the above mentioned substances was found to be almost the same as that of the fresh, raw apples. In a few cases it seemed that the permeability had slightly increased by the heating but the differences were so slight that they may very properly be attributed to individual differences.

Low temperatures were also used on Black Ben Davis apples, which were exposed to a temperature of -8°C . for 5 hours. The frozen apples were allowed to thaw out and come to a temperature of 20°C . when they were placed in the solutions used in the above experiments. In this case H_3BO_3 from saturated solution entered the apples in 50 hours and from 0.1 molar H_3BO_3 solution in 168 hours. Lithium was detected in the fruit immersed in 0.1 molar LiCl , LiNO_3 and Li_2SO_4 after 22 hours, also from saturated $\text{Li}_2\text{B}_4\text{O}_7$ after 22 hours. No KI was detected in the apples that had been frozen even after 118 hours and BaCl_2 was not found though the experiment ran for 168 hours.

Freezing the apples made it possible for most of the various substances to pass through the skins more readily and copiously than through the normal skins of the same variety of apples. The frozen apples immersed in the various solutions did not show the development of any decayed areas while in the liquid. This would indicate that no gross breaks or ruptures were made in the skins by the process of freezing, for such breaks are uniformly marked by a decaying of the fruit when in contact with the solutions.

Apples of the same variety were exposed to the vapors of boiling acetone for 20 to 30 minutes. The acetone removed most of the wax from the skins and also much of the coloring matter. In other experiments it was found that acetone was the only common solvent which removed the coloring matter, although all removed most of the wax from the skins.

The apples were allowed to cool and were then placed in the various solutions. After the treatment, H_3BO_3 entered from saturated solution in 26 hours very copiously, and $\text{Na}_2\text{B}_4\text{O}_7$ from 0.1 molar solution also entered very abundantly in 26 hours. Lithium entered the acetone treated apples from saturated $\text{Li}_2\text{B}_4\text{O}_7$ in 10 hours, and from 0.1 molar LiCl , LiNO_3 and Li_2SO_4 in 10 hours in very notable quantities. At the end of 26 hours, KI was found in the apples in large amounts and even BaCl_2 had entered by this time in notable quantities.

The boiling point of acetone is $56\text{--}57^\circ \text{C}$. It is not this temperature which caused the increased permeability, for the same variety of apples were exposed to a temperature of 75°C . without marked increase in the permeability of their skins. It consequently seems probable that the substances enter the apples more readily because the solvent has removed the waxy coating.

The Baldwin apples used for experimentation were obtained from a dealer who had obtained them from northern Michigan. The apples had never been in cold storage, but were wrapped individually in tissue paper and stored in a cool place in the basement of the chemistry building, from whence they were taken to the laboratory as needed.

In the Baldwin apples, H_3BO_3 from saturated solution could not be detected after 144 hours had elapsed, nor from 0.1 molar solution after 267 hours. From 0.1 molar solution, $\text{Na}_2\text{B}_4\text{O}_7$ was found in the apples after 146 hours. Lithium had passed into the apples from 0.1 molar LiCl in 80 hours and from 0.1 molar Li_2SO_4 in very small amounts in 50 hours; but 0.1 molar KI did not pass through the skins in 100 hours, the duration of the experiment. Barium chloride from 0.1 molar solution did not enter the Baldwin apples, although the experiment was continued for 312 hours. These apples were also immersed in 0.1 molar KCNS , but no trace of this salt could be found under the skin of the apple even after 122 hours of immersion. The skins of these apples were in general more permeable to the substances used than the Wealthy and Ben Davis apples, and less permeable than the skins of the Talent and Black Ben Davis apples. Each variety of apple, however, displays some peculiarities toward various individual reagents.

Baldwin apples were placed 18 inches from a mercury vapor lamp and were irradiated continuously for 2 hours. The odor of ozone was quite strong in the neighborhood of the lamp and the natural odor of the apples was much enhanced during and for a short time following the irradiation. After treatment, H_3BO_3 passed into the irradiated apples from saturated solution in 80 hours and from 0.1 molar solution in 267 hours, while from 0.1 molar solution $\text{Na}_2\text{B}_4\text{O}_7$ entered in 80 hours. Lithium was detected in the apples from 0.1 molar LiCl in 22 hours and from 0.1 molar Li_2SO_4 in

34 hours, and 0.1 molar KI entered the irradiated fruit in 80 hours. In every solution used, the solute passed through the skins of the apples which had been exposed to ultra-violet light for 2 hours much more rapidly and copiously than through the skins of the untreated fruit. These results are quite different from those obtained with the irradiated Wealthy apples.

To determine what effects ozone itself might produce, Baldwin apples from the same lot used for the above experiments were exposed for 20 minutes to an atmosphere of oxygen and ozone containing 2.25 grams of ozone per cubic meter of mixture. The ozone was produced by the action of the silent electric discharge on dry oxygen. After a short exposure to ozone, the natural odor of the apples was found to be much enhanced. For a few minutes after the apples were removed from the atmosphere of ozone, their waxy coating was very soft and sticky, and the subsequent hardening of the wax took place gradually. The apples were not placed in the solution until after the wax had hardened. Within a few hours after the treatment with the ozone-oxygen mixture, brown spots appeared at the lenticels of the apple skins. These small openings or pores could be seen with the naked eye in the normal fruit and it was evident that they were the locations at which the ozone took hold most strongly. It was shown in the case of the KI solution that it was only at these points or areas that the solution entered. Iodine could be detected in the tissue immediately under the spots, but at no other place in the tissue.

Boric acid from saturated solution entered the Baldwin apples treated with ozone in 24 hours and from 0.1 molar solution in 90 hours; lithium passed into the fruit from 0.1 molar LiCl in 10 hours and from 0.1 molar Li_2SO_4 in 20 hours; and KI was found under the affected spots after 42 hours. From 0.1 molar solution, BaCl_2 did not enter the apples although the experiment was continued for 312 hours. These results show that a very marked increase in the rate and extent of entrance of the various solutions used is caused by the action of ozone on the skins of the apples.

Finally, the effects of peroxide of hydrogen were studied. Baldwin apples were treated with H_2O_2 prepared by passing ozone into water and also by the action of H_2SO_4 on an excess of BaO_2 . The concentration of the H_2O_2 ranged from 0.1 per cent. to 3.0 per cent. by weight. The apples were immersed in these solutions for 30 minutes and then placed in the various solutions, but no increased permeability was noted due to the action of H_2O_2 . By using more dilute ozone the effect which that substance produces is naturally greatly lessened. It is still a question whether the increased permeability of the skins of irradiated apples is due to ozone formed by the ultra-violet rays.

The remarkable fact was observed that apples treated with H_2O_2 were much less permeable to Li_2SO_4 than the untreated apples, requiring 192

hours before lithium could be detected. The effect of the ozone on the apple skins was evidently due to some action of its own, therefore, and not to that of H_2O_2 formed by its reaction with the moisture present on the surface of the fruit.

Summary

1. Various fruits and vegetables were immersed in solutions, and the rapidity and copiousness with which the substances passed through the outer membranes determined. The solutions used were 0.1 molar and in a few cases saturated solutions of LiCl , LiNO_3 , Li_2SO_4 , $\text{Li}_2\text{B}_4\text{O}_7$, H_3BO_3 , $\text{Na}_2\text{B}_4\text{O}_7$, KI , BaCl_2 and SrCl_2 . Other solutions were used in a few experiments.

2. The skins on unripe sour cherries were found to be more permeable than the skins of ripe fruit from the same tree. Substances did not pass through the leaves and into adjacent cherries when the leaves were immersed in the solutions. California cherries burst before any of the substances in the solutions could be detected in the fruit.

3. Red Wisconsin plums were more permeable than either blue or yellow California plums. The permeabilities of the blue and yellow plums were about the same. The three varieties frequently burst during immersion, but without increase of weight.

4. LiCl , Li_2SO_4 and LiNO_3 were the only substances which passed through the skins of freshly picked gooseberries readily and copiously.

5. California grapes were found to be more permeable than Wisconsin grapes. The latter were found to be practically impermeable to all of the substances used except LiCl , LiNO_3 and Li_2SO_4 , which passed through after long immersion. Irradiation with ultra-violet light did not cause increased permeability of the skins of the grapes.

6. Toward LiCl , LiNO_3 and Li_2SO_4 the skins of green tomatoes were less permeable than the skins of ripe tomatoes. The reverse was true for H_3BO_3 , $\text{Na}_2\text{B}_4\text{O}_7$ and $\text{Li}_2\text{B}_4\text{O}_7$. The skins of the tomatoes were the only membranes investigated which were more readily permeated by H_3BO_3 and KI than by LiCl , LiNO_3 , and Li_2SO_4 .

7. All the substances used entered cucumbers and carrots quite readily and copiously, in the latter case probably because of the formation of openings through the laceration of the rootlets when the carrots were removed from the soil.

8. The only substance used which did not readily pass through the skins of Elberta peaches was SrCl_2 . Some of the solutions used caused discoloration of the skin and tissues of the peaches.

9. Milton jelly crabapples, and Wealthy, Talent, Ben Davis, Black Ben Davis and Baldwin apples were found to show selective action toward the

various solutions used. Russet apples were found to be more permeable than the same variety without russet.

Irradiation with ultra-violet light and exposure to dilute ozone was found to cause increased permeability of the skins of apples to most of the substances tested, but H_2O_2 had practically no effect on the permeability of the membranes. Removal of the waxy coating from the skins caused a very marked increase in the rate and extent of entrance of substances into the fruit.

Freezing without rupturing the skins was followed by a more rapid and copious entrance of the substances, while on the other hand, heating to 75° C. had very little effect on the permeabilities of the membranes.

DEPARTMENT OF CHEMISTRY,
UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN

NITROGENOUS METABOLISM OF *PYRUS MALUS* L.

I. INFLUENCE OF TEMPERATURE OF DESICCATION ON WATER-SOLUBLE NITROGENOUS CONSTITUENTS AND SEPARATION OF WATER-SOLUBLE PROTEIN FROM NON-PROTEIN CONSTITUENTS¹

WALTER THOMAS

Introduction

Notwithstanding the fact that nitrogen occupies a unique position in plant metabolism, our knowledge concerning the processes of absorption, synthesis or ultimate disposition of its compounds in the metabolic cycle of the higher plants is very limited.

The various groups of the complex combinations which nitrogen is capable of forming may have quite different physiological functions and, accordingly, as SPOEHR and MCGEE (23) have already emphasized, it is improbable that a rational conception of the function of nitrogen in the higher plants can ever be obtained without further studies of the physiological rôle of its various groups. Thus, a knowledge of the part these groups play and the extent to which their reactivity can be controlled are questions that must be solved before the problems of growth, reproduction and movement can be solved.

Owing to the very large number and complexity of the compounds which nitrogen forms, the separation and identification of its various groups or compounds are beset with difficulties; but the advances made in the chemistry of proteins have paved the way for an approach to investigations on nitrogen metabolism that were not available to the older workers in this field (9).

The possibilities which lie in the acquisition of greater knowledge of these weighty problems by the more modern method of approach are indicated by Chibnall's (2) studies on the distribution of nitrogen in the leaves of the Runner Bean (*Phaseolus vulgaris*) throughout a vegetative cycle;

¹ Published by permission of the Director of the Agricultural Experiment Station as scientific paper no. 418 and contribution no. 24 from the Department of Agricultural and Biological Chemistry.

The cost of these investigations, forming a part of a cooperative project with the Department of Horticulture, has largely been defrayed by a grant from the Adams Fund for Agricultural Research.

but no investigations have been reported on the distribution or partition of nitrogen in the tissues of fruit trees throughout a cycle.

The present studies were undertaken for the purpose of obtaining data that might give an insight into the transformations taking place, as measured by the nitrogen fractions soluble in water, at the different vegetative periods throughout a year's cycle. When the possibilities and limitations of such studies are known it should then be possible to determine to what extent such information might be utilized, more especially in relation to the problems of growth and reproduction. The problem necessarily involves the simultaneous study of the validity of methods.

A. THE EFFECT OF TEMPERATURE OF DESICCATION ON THE WATER-SOLUBLE NITROGENOUS CONSTITUENTS

As the nature of the problem necessitated the accumulation of samples for subsequent analyses, prevention of enzymatic activity as soon as possible after the samples are collected is essential. Preservation by means of alcohol is undesirable because of its precipitating effect on the proteins. Freezing also produces marked changes in some plants (27). The available evidence indicates that inactivation of proteolysis by dehydration by drying probably causes less changes in the tissues than any of the methods at present in use. The question of the optimum temperature at which autolytic changes are reduced to a minimum has received attention recently by TOTTINGHAM, SCHULZ and LEFKOVSKY (27) and by LINK and SCHULZ (17). These papers and also a paper by CHIBNALL (3) contain a discussion (with references) of the temperatures adopted by previous investigators for the desiccation of the tissues of various plants. None of the earlier workers appear to have made any investigation into the effects of temperature on the composition of the plants under experiment.

Although CHIBNALL (3) found, in the case of the leaves of the Runner Bean, that low-temperature drying in a closed oven at 40–50° C. entailed protein autolysis, causing an increase in ammonium salts, asparagine and amino acids, the American investigators (17, 27), under the conditions adopted by them, found that the greatest changes produced either by rapid drying or freezing resulted from the coagulation of soluble proteins. Coagulation was greatest at high temperatures; at low temperatures (32–40° C.) both coagulation and proteolytic changes seemed to occur.

Experimental

The material used for this investigation was obtained from Stayman Winesap apple trees 15 years old growing in the College orchard. Samples of leaves and new shoot growth were collected in the early part of a July morning when active growth was in progress.

TEMPERATURES ADOPTED IN THIS INVESTIGATION

The experiences of previous investigators (17, 27) that very high and very low temperatures are to be avoided, minimum changes occurring at "intermediate" temperatures (50–70° C.), afford a sufficient basis for the selection of the three temperatures, 50, 60 and 70° C., for the determination of the optimum temperature causing minimum proteolytic changes in the water-soluble nitrogenous constituents of the species under investigation.

At 50 and 60° C. desiccation was carried out in ordinary Freas ventilated ovens. Drying at 70° C. was accomplished in wire bottom trays in a drying closet heated by steam coils. To further restrict the possibility of enzymatic activity the moisture content was reduced to 0.5 per cent. by transference to a vacuum oven for eight hours after the samples had been ground. The samples were preserved in glass stoppered sealed bottles. A month elapsed before analytical work was commenced on the dried samples.

PREPARATION OF SAMPLES FOR ANALYSIS

The removal of the vacuole and colloidal cytoplasmic cell contents from the fresh leaves was readily accomplished by means of a Nixtamal mill (19) which readily disintegrates the cells of succulent plant materials after two or three passages through the mill. However, the disintegration of the cells of the refractory woody shoot growth could not be accomplished by this means. This was finally effected by the laborious process of alternate passage through an Enterprise mill and mincing by hand and afterwards macerating with quartz sand in the manner to be described later. The dried material was easily ground by a method previously described (26).

METHOD OF EXTRACTION OF THE SOLUBLE NITROGENOUS CONSTITUENTS: DIRECT METHOD VERSUS CHIBNALL'S PROCEDURE

TOTTINGHAM, SCHULZ and LEPKOVSKY (loc. cit.) found the direct extraction with water to be a satisfactory method for partitioning the products of the nitrogenous constituents of barberry and sugar beet leaves. Water enabled them to extract the greater part of the proteins exposed by crushing the cells of the fresh tissues, the direct method compared with CHIBNALL'S (4, 5) giving somewhat higher values for total water-soluble nitrogen.

Comparison of the direct method with CHIBNALL'S method by the writer on apple leaves indicated that only two-thirds as much protein N and non-protein N were found in the extracts obtained by the latter method. These results would indicate that the modifying action of the cytolytic agent (ether) in CHIBNALL'S procedure is of greater influence than the post-mortem changes due to the "setting" of the cytoplasm into a gel (6).

All extractions were made by grinding 50 to 100 gm. of the material in a mortar with water (about 200–300 cc.) containing 20 cc. of 2 per cent. phenol. The brownish red extract was decanted on a filter of paper pulp, using a Buchner funnel, and the residue, after washing with water, again transferred to the mortar for further grinding. The process of filtering and grinding was repeated until microscopic examination showed that 85–90 per cent. of the cells were broken up. All samples were made up to a volume of 2,200 cc. and covered with a layer of toluene.

METHODS OF ANALYSIS

The methods of determining the various fractions will be given in detail in the third paper of this series in connection with the partition experiments.

TABLE I

TOTAL WATER-SOLUBLE N, NON-PROTEIN N, AMMONIA N AND AMIDE N IN THE LEAVES
a. AS PERCENTAGES OF THE DRY MATTER

SERIES NUMBER	TOTAL N	TOTAL WATER SOLUBLE N	NON-PRO- TEIN N	AMMONIA N	AMIDE N
OA	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Fresh	1.80	0.130	0.089	None	0.014
Dried at 50°	1.82	0.120	0.090	None	0.016
Dried at 60°	1.82	0.120	0.092
Dried at 70°	1.83	0.109	0.098	None	0.016
18					
Fresh	1.86	0.146	0.120	0.003	0.013
Dried at 50°	1.86	0.144	0.124	0.006	0.017
Dried at 60°	1.85	0.144	0.123	0.006	0.017

b. AS PERCENTAGES OF THE TOTAL NITROGEN OF THE DRY MATTER

SERIES NUMBER	TOTAL N	TOTAL WATER SOLUBLE N	NON-PRO- TEIN N	AMMONIA N	AMIDE N
OA	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Fresh	1.80	7.22	4.94	0.77
Dried at 50°	1.82	6.59	4.94	0.88
Dried at 60°	1.82	6.59	5.05
Dried at 70°	1.83	5.95	5.35	0.87
18					
Fresh	1.86	7.85	6.45	0.16	0.70
Dried at 50°	1.86	7.74	6.66	0.32	0.91
Dried at 60°	1.85	7.59	6.65	0.32	0.91

In the present experiments the effects of desiccation on the sap contents was followed by observation of the changes in the total water-soluble N, non-protein N, ammonia N and amide N.

The results obtained are given in tables I and II.

TABLE II

TOTAL WATER-SOLUBLE N, NON-PROTEIN N, AMMONIA N AND AMIDE N
IN THE NEW GROWTH

a. AS PERCENTAGES OF THE DRY MATTER

SERIES NUMBER	TOTAL N	TOTAL WATER SOLUBLE N	NON-PRO- TEIN N	AMMONIA N	AMIDE N
OB	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Fresh	0.715	0.163	0.099	0.003	0.009
Dried at 50°	0.715	0.155	0.110	0.004	0.014
Dried at 60°	0.711	0.155	0.104
Dried at 70°	0.710	0.137	0.103	0.004	0.011

b. AS PERCENTAGES OF THE TOTAL NITROGEN OF THE DRY MATTER

SERIES NUMBER	TOTAL N	TOTAL WATER SOLUBLE N	NON-PRO- TEIN N	AMMONIA N	AMIDE N
OB	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Fresh	0.715	22.8	13.8	0.419	1.250
Dried at 50°	0.715	21.6	15.3	0.559	1.950
Dried at 60°	0.711	21.8	14.6
Dried at 70°	0.710	19.3	14.5	0.563	1.549

Discussion

These results, as also those of TOTTINGHAM and his co-workers (27), indicate that the higher the temperature (within limits) the greater becomes its influence on the coagulation of soluble proteins. In the case of the woody branch growth this appears to be accompanied by greater proteolytic changes at the lowest temperature (50° C.), a possible explanation of which is the longer time required for the desiccation of ligneous tissues.

It is apparent, however, that desiccation of the tissues of this plant species, which is low in water-soluble nitrogen, under the temperature conditions described, does not greatly change the proportions of total water-soluble N and non-protein N and that the changes in the ammonia N and amide N are not, except in the woody tissues, much greater than the error of determination. The optimum temperature appears to be around 60° C.

These findings and those of LINK and SCHULZ (17) on the barberry suggest that the proteolytic enzymes, due possibly to the different nature of the nitrogenous or carbohydrate constituents, may be relatively less active in plants low in water-soluble nitrogen than in those that are relatively high in these constituents. But whether, as suggested by LINK and SCHULZ, the differences observed between the high and low water-soluble nitrogen types of plants are due to the initial water content and the rate of its removal can, it seems to the writer, only be determined by further investigations in which plants low in the soluble cytoplasmic proteins are compared with those that are high (7). Until such questions are cleared up each plant tissue must represent a separate problem, in which case generalizations extended to all types of tissues are impossible.

B. THE SEPARATION OF THE PROTEINS FROM THE NON-PROTEIN CONSTITUENTS

Although, for the accurate determination of the amino nitrogen content, the separation of the proteins from the other nitrogenous constituents of the extracts of plant cells is by some investigators (24) considered unnecessary, others (28) have shown the advantage of doing so. The water-soluble protein in the plant species under investigation is, as we have seen, quite low; nevertheless, apart from the desirability of having some knowledge concerning its amount and fluctuations during the vegetative cycle, positive evidence was obtained that the removal of the soluble protein by the method to be described herein enabled more consistent results to be obtained in the estimation of the several non-protein fractions with which these metabolism investigations are mainly concerned.

The objective is to effect the separation with the removal of as little as possible of the intermediate products of protein degradation; but whether any reagent accomplishes the complete exclusion of all products which are only a little below the original proteins in their complexity from the protein-separates is doubtful.

NOTES ON THE REAGENTS USED IN THIS INVESTIGATION

Since STUTZER (25) proposed copper hydroxide for the removal of "peptides" from the "amide N," a number of reagents, such as methyl alcohol, acetic acid, trichloroacetic acid, phosphotungstic acid, tannic acid, and colloidal ferric hydroxide, have been used for the separation of the "protein" from the "non-protein" constituents, and, unfortunately, the evidence points to the conclusion that a reagent satisfactory for one kind of biological material may be unsuitable for another.

HART and BENTLEY (13) have shown that STUTZER's reagent may not, in all cases, effect a complete separation of all protein and polypeptide

structures from amines and amides, for, although the copper salts of a number of di-, tri-, and tetra-peptides have been prepared, having the formula (peptide)₂:Cu (16), not all peptides may form insoluble copper salts under the conditions usually adopted for the precipitation. From the standpoint of the present investigations the chief objection to the use of copper salts is that under the conditions adopted they form insoluble copper compounds with certain amino acids.

BLISH (1), working on wheat flour extracts, following a suggestion by OSBORNE and LEAVENWORTH (20), obtained very nearly complete separation. He treated the extracts with 0.1 N NaOH followed by 0.1 N CuSO₄ until the latter was only in slight excess of the equivalent required. Although his precipitates were free from amino groups, evidence was presented that peptide linkings of unknown nature but of less complexity than true proteins were still present in small amounts in the filtrates.

Both acetic acid and trichloroacetic acid are used by a large number of investigators both in the animal and plant physiological fields. The use of the latter was proposed by GREENWALD (11) because of its easy solubility, its ready volatility and its non-solvent action on proteins when used in excess. It is claimed that it does not precipitate either the proteoses or the peptones (12), although the results obtained in this investigation are inexplicable on this basis.

Colloidal ferric hydroxide was first introduced by RONA and MICHAELIS (22), since which time it has received more favor as a precipitant for blood protein than possibly any other reagent. The evidence presented by CLEMENTI (8) and also by VAN SLYKE, VENOGRAĐ-VILLCHUR and LOSEE (29) indicates that this reagent precipitates none of the amino acids; the latter also found that it precipitates none of the intermediary products up to the proteoses and none of these except some of complexity but little below that of the original proteins. Moreover, since the present investigations were completed, FODOR and REIFENBERG (10) have shown that the proteins adsorbed by the ferric hydroxide gel can be recovered completely by eluting the residuum with NaOH.

Mercuric salts have the advantage that they remove all pigments, giving colorless solutions; but the excess of mercury is not conveniently and easily removed. Like copper they also precipitate polypeptides.

Experimental

The effects of four of these reagents on the nitrogenous constituents of the water extracts of the leaves of *Pyrus Malus* were examined. The technique of precipitating the soluble proteins with the various reagents was as follows:

Acetic acid and trichloroacetic acid.—The extract (2,000 cc.), after the addition of 10 cc. of 10 per cent. acetic acid or of 25 cc. of a 10 per cent. solution of trichloroacetic acid, was heated to boiling and boiled one to two minutes. The coagulum was separated by a fluted filter paper, washed with 100 cc. hot water, and made up to the original volume.

Copper hydroxide.—To the extract, measuring about 2,000 cc. in a 4 liter flask, was added 400 cc. of a freshly made-up solution of 0.2 N NaOH, followed by 400 cc. of 0.2 N CuSO_4 . Ten cc. portions of the 0.7 N CuSO_4 were then added, and after each addition the extract was well shaken and the precipitate allowed to subside. The copper-protein precipitate was removed by filtration and washed with water.

Colloidal ferric hydroxide.—Merek's colloidal ferric hydroxide was used. The extracts were brought just to the boiling point, when an excess of the reagent, determined by examining the filtrates for freedom from proteins, is added. In the present experiments 5–6 cc. is sufficient. The extracts were then brought to the neutral point and the boiling continued for one or two minutes. In partition experiments of the nature to be described, it is necessary to remove the excess of iron by the addition of MgSO_4 or some other salt of high coagulating power. From 0.5 to 1.0 cc. of a saturated solution of this salt is sufficient to produce coagulation of the iron gel. The precipitate of protein-ferric hydroxide gel is separated by filtration, the precipitate washed, and the filtrate made up to the original volume.

DETERMINATION OF WATER-SOLUBLE PROTEIN NITROGEN

In all cases the protein N was determined by difference between the non-protein N and the total water-soluble N. The large fluted filter necessary for the filtering required too large a blank to enable the small amount of protein to be determined directly with any degree of accuracy.

In table III the mean of closely agreeing duplicate determinations is given.

TABLE III

COMPARISON OF PROTEIN N, NON-PROTEIN N AND AMINO N IN THE WATER EXTRACTS

SERIES NO. OC	NITROGEN IN THE DRY MATTER				
	TOTAL N	TOTAL WATER SOLUBLE N	PROTEIN N	NON-PROTEIN N	AMINO N
PRECIPITATING REAGENT	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
	1.83				
Acetic acid	0.144	0.034	0.110	0.014
Trichloroacetic acid	0.146	0.034	0.112	0.018
Copper hydroxide	0.146	0.078	0.038	0.008
Colloidal ferric hydrate..	0.142	0.022	0.120	0.024

QUALITATIVE TESTS ON THE FILTRATES FROM THE VARIOUS PRECIPITATING REAGENTS

The filtrates from the various reagents used for removing the proteins were subjected to the biuret, Millon and Adamkiewicz reactions. Such tests serve as a general guide, but caution must be used in drawing conclusions, for, as MOLISCH (18) has pointed out, the blue color of the biuret test is given also by certain carbohydrates and a number of aliphatic acids; hence, the test is not specific for proteins and the $-\text{NH}_2 - \text{CO} - \text{CO} - \text{NHR} -$ groups. Neither can the Millon test be regarded as specific for the tyrosine and tryptophane groups of proteins, because it is also given by phenol, salicylic acid and other substances containing a monohydroxybenzene nucleus, upon the presence of which the test for proteins depends. The biuret test is best carried out by the method of KANTOR and GIES (15), who describe the preparation of a stable biuret reagent which is more sensitive than the classical method of application. The Adamkiewicz reaction was found in the present investigation to be more sensitive if the solution to be tested is concentrated almost to dryness *in vacuo*, followed by extraction with glacial acetic acid and concentrated HCl and not concentrated H_2SO_4 as usually recommended.

Some investigators (10) use SPIEGLER and ESSBACH's reactions to differentiate the derived from the non-derived proteins (*i.e.*, the simple and conjugate proteins free from their hydrolytic products). However, it is questionable (14) if these reagents are specific.

One of the difficulties encountered in applying such tests to plant extracts is the difficulty in removing all pigments present without also removing some of the products to be tested. The present tests were made on carbon black-treated extracts, which were concentrated *in vacuo* after filtering.

The results are conveniently shown in table IV.

TABLE IV
QUALITATIVE TESTS OF EXTRACTS FOR PROTEINS

REAGENT	FILTRATES	BIURET	ADAMKIEWICZ	MILLON
Copper salt	clear	negative	negative	slight positive
Acetic acid	turbid	positive	positive	positive
Trichloroacetic acid	turbid	positive	positive	negative
Colloidal ferric hydroxide	clear	positive	positive	positive

Qualitative tests, however, although devised for pure proteins and other decomposition products, may throw additional light on the results obtained by quantitative means and thus serve as an aid in interpretation.

Discussion

The quantitative results, table III, show that CuSO_4 and NaOH have removed more nitrogen from solution than the other reagents. This appears to be the result of the occlusion or formation of insoluble copper-amino-acid compounds (*e.g.*, asparagine) as well as to the complete removal of the specific peptides occurring in these extracts together with products intermediate between these and the true proteins. The negative biuret reactions in the copper filtrates give further confirmation of this, for a positive test is first encountered in certain tetrapeptides and is the more intense the greater the length of the polypeptide chain (21). This same test shows that the other reagents have not removed peptides and the intermediate products. The colloidal ferric hydroxide gel contains the lowest amount of nitrogen and the highest quantity of amino N. The presence of proteose N in the filtrates from the colloidal iron treatment may be objectionable from the standpoint of its influence on the determination of amide N, because of the possible error in the determination of amide N in the process of hydrolyzing the extracts. This was not investigated further because the amounts present are so small that the effect on the analytical results would be insignificant. The evidence to be presented in the third paper of this series shows clearly that all filtrates contain the CONH groupings.

Colloidal ferric hydroxide, then, is admirably suited for the separation of true proteins from solution because: (1) It is more convenient and expeditious; (2) it permits all the amino acids to go through, occluding and precipitating none; (3) it effects sharp separation of true protein from its decomposition products; and (4) the adsorbed proteins can be recovered quantitatively from the residuum.

Summary

Desiccation of the leaves and new wood growth from apple trees at 50°, 60° or 70° C. has no effect on the total nitrogen; but relatively small decreases in the soluble cytoplasmic proteins, and increases in the non-protein constituents, occur through drying. Considering the effect of temperature on both coagulation and proteolysis, it would appear that a temperature of 60° C. is the optimum for the desiccation of the tissues of this species, which belongs to the type of plants that are low in water-soluble protein nitrogen.

Colloidal ferric hydroxide has many advantages over the reagents in general use for the separation of the simple and conjugate proteins from their hydrolytic products. Reasons for this choice are given.

The writer takes this opportunity of expressing his thanks to Professors J. B. HILL and L. O. OVERHOLTS for microscopic examination of some of the extracted materials.

DEPARTMENT OF AGRICULTURAL AND BIOLOGICAL CHEMISTRY,
AGRICULTURAL EXPERIMENT STATION,
THE PENNSYLVANIA STATE COLLEGE.

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NITROGENOUS METABOLISM OF *PYRUS MALUS* L.

II. THE DISTRIBUTION OF NITROGEN IN THE INSOLUBLE CYTOPLASMIC PROTEINS¹

WALTER THOMAS

Before undertaking the experimental work on the distribution, throughout a year's cycle, of the soluble nitrogenous constituents of the leaves and branch growth, which will be presented in the third paper, it was considered of interest to obtain information respecting the nature of the insoluble leaf proteins and to ascertain what fluctuations, if any, occur during development, in the distribution of its nitrogen groups (*i.e.*, its amino acid make-up). An additional motive for a closer study of this insoluble portion was afforded by the relationships which NIGHTINGALE (8) seemed to find between his alcohol insoluble nitrogen fraction, and carbohydrates, in the vegetative and reproductive processes of the tomato plant (*Lycopersicum esculentum*).

Sufficient knowledge is not yet available to enable these insoluble proteins to be isolated in a state of purity, but VAN SLYKE (11, 12) has devised a method for determining the distribution of nitrogen in the various groups of the protein molecule which has furnished means of classifying different proteins by their amino acid make-up. This method was, however, devised for pure proteins and their hydrolytic products (1, 2, 3), but as MORROW and GORTNER (7) have pointed out, the extension of the method may be made to impure proteinaceous substances when due precautions are taken and no attempt is made to assign any part of the nitrogen to any specific amino acid.

Experimental

Samples of leaves were collected, from a 15-year-old Stayman Winesap tree growing in sod, at three critical physiological periods, *viz.* (1) at the early period of bud formation (May 13), (2) at the stage of active maximum growth (July 22), and (3) at the period of chlorophyl degeneration, *i.e.*, of declining metabolic activity (Nov. 11).

Five grams of each residuum, dried *in vacuo*, remaining after the extraction with water as described in the preceding paper and consisting, therefore, of the insoluble cytoplasmic proteins and the cellulose material of the cell wall, together with such non-protein nitrogenous materials as fats, lecithins, chlorophyl, and the purine and pyrimidine bases, were ex-

¹ Published by permission of the Director of the Agricultural Experiment Station as scientific paper no. 419 and contribution no. 25 from the Department of Agricultural and Biological Chemistry.

tracted with alcohol followed by ether for the removal of fats, chlorophyll and lecithins, and afterwards digested with 0.5 per cent. HCl (10) to hydrolyze the interfering carbohydrates (5). After filtering and washing with hot water, each residuum, consisting of the impure proteins, was hydrolyzed with 20 per cent. HCl for 48 hours on a sand bath, with the usual precautions, to insure comparable results (4). The small amount of protein N in the filtrate from the 0.5 per cent. HCl hydrolysis was separated and hydrolyzed after the process described by HAMILTON, NEVENS and GRINDLEY (5). After the removal of the HCl by distillation *in vacuo*, the two hydrolysates were combined. The residue was dissolved in 100 cc. of water and a slight excess of MgO added. The process of determining the nitrogen fractions was then, with slight modifications, similar to that described by MORROW and GORTNER (7).

The analytical data are presented in table I.

TABLE I
THE INSOLUBLE CYTOPLASMIC PROTEINS
DISTRIBUTION OF N AS PERCENTAGE OF TOTAL NITROGEN

NITROGEN FRACTION	SERIES NO. 3		SERIES NO. 20		SERIES NO. 33	
	(a)	(b)	(a)	(b)	(a)	(b)
Ammonia N.....(A)	15.50	15.20	14.80	14.20	16.40	16.00
Insoluble humin N.....(B)	7.82	8.00	7.75	7.70	9.00	8.80
Humin N precipitated by Ca(OH) ₂(C)	0.23	0.20	0.24	0.27	0.24	0.29
Total humin N.....(A + B)	8.05	8.15	7.99	7.97	9.24	9.19
Total basic N.....(D)	20.62	20.55	21.80	21.70	22.05	20.30
Basic N set free as NH ₃ by 50% KOH (arginine).....(E)	5.80	lost	5.92	6.00	6.34	6.15
Basic N not set free as NH ₃ by 50% KOH.....(D - E)	14.82	15.88	15.70	15.71	14.15
Amino N of bases.....(F)	5.25
Non-amino N of bases.....(D - F)	15.37
N in filtrate from bases.....(G)	53.10	52.80	54.85	54.91	55.00	54.20
Amino N in filtrate from bases.....(H)	51.30	51.00	53.20	53.00
Non-amino N in filtrate from bases.....(G - H)	1.80	1.80	1.65	2.00
Total N obtained.....	97.27	98.72	99.44	102.69	99.80

In series no. 33 (a) fats, lecithins, chlorophyll, etc., were removed by previous extraction with alcohol and then with ether. This would contain a small amount of nitrogen, but the total amount as compared with the protein N present is small.

Discussion and conclusion

Is it justifiable to draw from the constancy of the distribution of nitrogen the conclusion that, although the leaf proteins vary quantitatively through-

out their cycle, they do not vary qualitatively? In other words, do these results indicate that a single protein exists in the leaves or do they contain a mixture of several? OSBORNE (9) believes that even if several proteins occur in these leaves they may be so nearly alike in their amino acid make-up that changes in their relative proportions can not be established by methods at present available. He is of the opinion that, though the above results do not give *absolute proof*, perhaps, that a single protein is present in these leaves, they do *indicate* that this is the case.

Even if these "insoluble" proteins could be isolated in the pure condition so that not only the percentage of amino acids but also their optical properties, molecular weight and elementary composition could be determined, the similarity of all these would not constitute indisputable evidence that the proteins were identical. If marked differences occurred in the amino acid content there would be little question as to the existence of a mixture of substances of unlike chemical entities; but these results, until new methods of study become available that enable the question of the true chemical entity of the proteins to be determined, do indicate that little, if any, qualitative change in the nature of the insoluble leaf protein has taken place during its development.

From this standpoint, then, it is clear that any relationship to plant performance that may be discoverable between the insoluble proteins and carbohydrates must be due to quantitative and not qualitative differences in the nature of the insoluble proteins.

DEPARTMENT OF AGRICULTURAL AND BIOLOGICAL CHEMISTRY,
AGRICULTURAL EXPERIMENT STATION,
THE PENNSYLVANIA STATE COLLEGE.

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SOIL-MOISTURE CONDITIONS IN RELATION TO PLANT GROWTH

F. J. VEIHMEYER AND A. H. HENDRICKSON

(WITH SIX FIGURES)

Recent investigations at the University of California have yielded much information regarding the relation of soil moisture to the normal growth and behavior of fruit trees, and have indicated serious disagreements with results secured by some previous investigators of the problem of water relations of plants. It is logical to suppose that the moisture conditions of the soil do not exert any direct retarding influence on the plant so long as water is supplied to the absorbing surfaces of the roots and is conducted to the leaves as rapidly as required by the transpiration rate. However, just as soon as the rate of supply falls below this requisite amount, then soil moisture becomes a limiting condition. An inadequate moisture supply may be evidenced by lessening of turgor, cessation of growth, and wilting, and, in more advanced stages, by death of the tissues. Of course, the wilting of a plant does not indicate that water has ceased to move from the soil into the plant, but simply that transpiration has exceeded absorption and conduction. It is obvious, since wilting is progressive, that various stages of wilt might be recognized. Several attempts have been made to fix upon a definite degree of wilting. The "saturation deficit" of RENNER (6) and the "incipient drying" of LIVINGSTON and BROWN (4) are not definite stages, but represent broad ranges in the progress of wilting, but the "permanent wilting" of BRIGGS and SHANTZ (1) represents a fairly definite stage or degree of wilting, which can be readily recognized in experimentation, and this has received much study. However, KOKETSU (3) has pointed out that definite stages of wilting may be recognized readily in *Mimosa* because of the movements of its leaves.

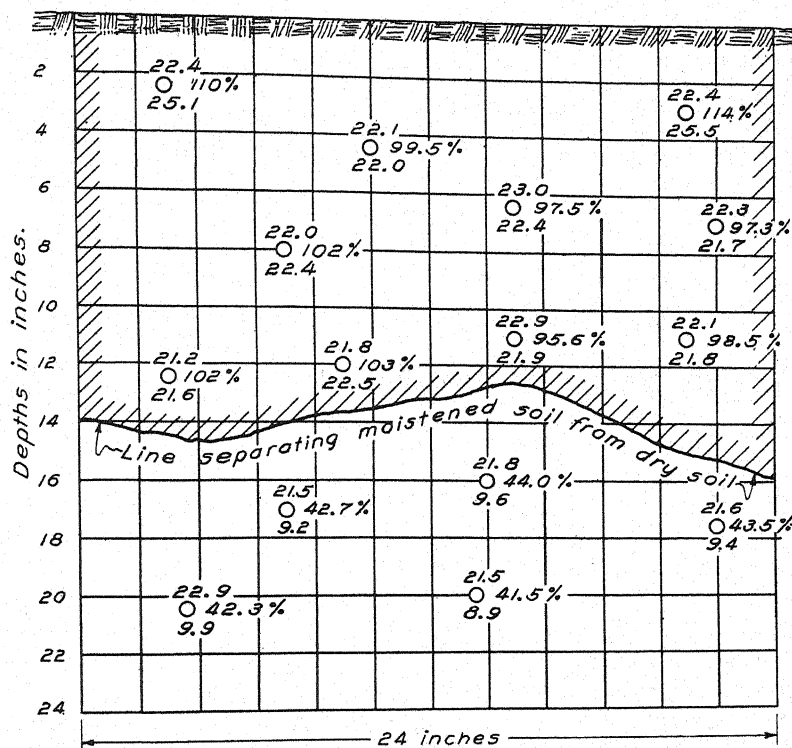
BRIGGS and SHANTZ defined their permanent wilting as that stage of wilting when the leaves first undergo a permanent reduction of their moisture content as a result of deficiency in the soil-moisture supply. A permanent reduction is here taken to mean a deficiency in leaf-water content from which the leaves do not recover in an approximately saturated atmosphere, without the addition of water to the soil. LIVINGSTON and KOKETSU (5) have further discussed the condition of permanent wilting and have emphasized the dynamic nature of the wilting process.

BRIGGS and SHANTZ (1) concluded from their studies that atmospheric environmental conditions have little or no effect upon the residual water content of the soil at the time of the beginning of permanent wilting and

that this residual water content, called by them the wilting coefficient, for any given soil is a constant for all species of plants grown on it and for all stages of their development. On the other hand, CALDWELL (2) and SHIVE and LIVINGSTON (8) found, from a study of plants grown in small containers, that the time required for the onset of permanent wilting after watering had been discontinued, and also the residual water content of the soil when permanent wilting began was dependent upon the intensity of the evaporation conditions for the period during which permanent wilting was attained. LIVINGSTON and KOKETSU (5) found that the water-supplying power of the soil was practically constant for all soils when permanent wilting began in their plants, and they predicted that this critical value of the supplying power would be found to depend upon the evaporation conditions.

Observations extending over a number of years in deciduous fruit orchards in California, as well as in experimental plots at Davis, California, at the Branch of the College of Agriculture of the University of California, indicate that the soil-moisture supply may fluctuate between wide limits without measurably affecting the growth of the tree or the yield and quality of the fruit. This range of fluctuations in the moisture of the loam soils on which the experiments were conducted was between the maximum field capacity and the calculated wilting coefficient, a range of 10 per cent. or approximately 9.5 acre-inches of water in six feet of soil. The difficulty of adequately sampling the soil in the orchard plots, in spite of the employment of an unusually large number of samples, and the consequent difficulty of determining with satisfactory certainty the average soil-moisture condition in the root zone, led to a study of trees grown in large containers, and the results from these experiments were substantially the same as those obtained in the orchard plots.

Experimental results based on the idea that water applied at any point in the soil would be quickly and uniformly distributed throughout the surrounding soil have led, in the opinion of the writers, to many erroneous conclusions. This thought has also recently been put forth by others, notably SHANTZ (7), who stated upon theoretical considerations that "Because of these peculiarities in the distribution of moisture in soils much of the work . . . is entirely unreliable and will have to be repeated when the conditions are known or better understood." The writers have been unable to maintain any soil-moisture content lower than that which the soil would hold against the force of gravity—the maximum field capacity. What takes place when water is applied to the surface of nearly dry soil is illustrated by the results of a test, the results of which are shown in figure 1. A rain of 2.15 inches on the surface of a loam soil resulted in wetting the soil to a uniform depth of about 14 inches, but no farther, samples being taken 48



○ = Point from which soil samples were taken.
 Upper numbers = Moisture equivalent determined by centrifuge method.
 Lower numbers = Actual moisture content of sample from field.
 Percentages are the ratios of the moisture content to the moisture equivalent.

FIG. 1. Vertical distribution of water in a loam soil 48 hours after a rainfall of 2.15 inches. The upper margin of the diagram represents the soil surfaces.

hours after the rain ceased. As the figure shows, the soil throughout the wetted region was raised to a moisture content closely approximating its moisture equivalent made by the method suggested by VEIHMAYER, ISRAELSON and CONRAD (9). In general, an application of a definite amount of water on the soil surface results in the wetting of the loam soils used in these experiments always just to a definite depth, this depth depending upon the water-holding capacity of the soil and its initial moisture content. This was observed many times in our work, both in field plots and in large con-

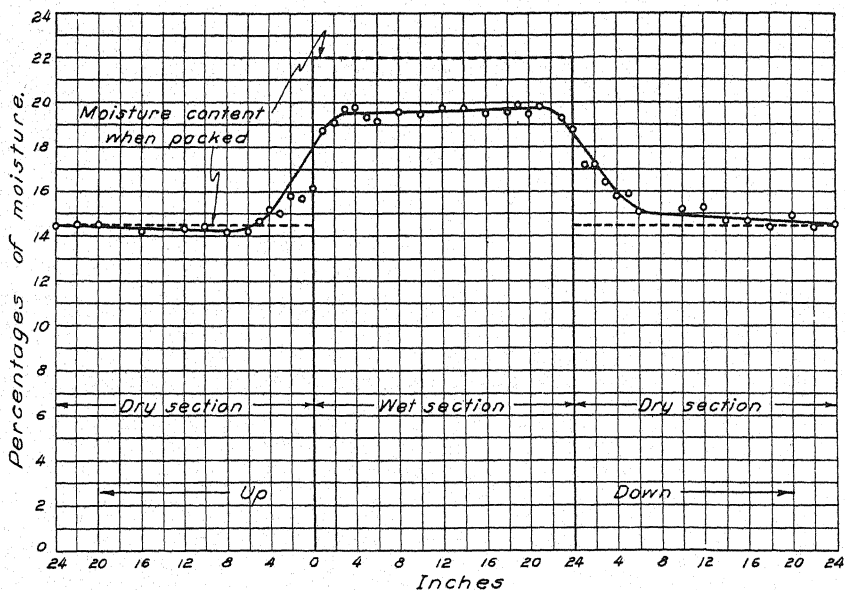


FIG. 2. The movement of moisture upward and downward (left and right in diagram) from soil mass initially containing 22 per cent. of moisture to soils containing 14.5 per cent. of moisture. Column was started September 2, 1922; samples were taken January 18, 1923. The place of sampling in the column and the amount of moisture found are indicated by the circles. Depth is shown by numerals along the base of diagram.

tainers. There is no evidence in such tests of the deeper soil being wetted at all by the surface application.

The results obtained in these experiments are contrary to a long accepted belief that water moves by capillarity with considerable speed from moist to drier soils. Such movement as occurs has been found in our tests to be extremely slow in rate and slight in both amount and extent. The data presented in figure 2 show the movement of moisture upward and downward within a period of 139 days, from a soil mass initially containing 22 per cent. of moisture, calculated on a dry weight basis, to soil masses above and below containing 14.5 per cent. of moisture. The column of loam soil had a cross-sectional area of 36 square inches. The central wet section was initially packed with soil containing water to the extent of its maximum field capacity (22 per cent.) and the upper and lower end sections were packed with the same soil but with moisture content somewhat above the calculated wilting coefficient. The extent of movement in either the upward or the downward direction was approximately 8 inches in the period of 139 days.

Like results were obtained in many similar trials, with soils of different initial moisture contents.

In the experiments with trees in containers, at each application of water the soil mass was raised to its maximum field capacity throughout. The trees were then allowed to deplete the average soil moisture to different extents and then water was again applied. The range of moisture fluctuations was slight, in some cases, while in others it was as much as from the maximum field capacity to the calculated wilting coefficient. These experiments continued through the growing season. The use of water by young French prune trees grown under these conditions of fluctuating soil moisture supply is shown by the data in table I.

TABLE I

AMOUNT OF WATER USED BY YOUNG FRENCH PRUNE TREES, AS RELATED TO LEAF AREA AND TO LENGTH GROWTH

NUMBER OF TREE	LENGTH GROWTH	NUMBER OF LEAVES	LEAF AREA	WATER USED MARCH 1 TO SEPTEMBER 25	RATIO OF WATER USE TO LEAF AREA (LOSS PER SQUARE INCH OF AREA)	RATIO OF LENGTH GROWTH TO WATER USE (INCHES OF GROWTH PER POUND OF WATER USED)
	inches		sq. in.	pounds	pounds	pounds
5	348.0	394	2098	499	0.237	0.698
12	214.0	239	1244	316	0.254	0.678
20	697.5	828	4305	1020	0.237	0.683
21	351.0	398	2070	508	0.245	0.690

For tree no. 5, the soil-moisture content was kept above 16 per cent. until the middle of August, being then alternately allowed to fall to approximately the wilting coefficient, and irrigated; for tree no. 12, the soil was kept water logged throughout the experiment; for tree no. 20, the soil-moisture content was maintained above 16 per cent.; and for tree no. 21, the soil-moisture content fluctuated between the maximum field capacity and an amount corresponding to the wilting coefficient. The maximum field capacity (or the moisture equivalent) for this soil was approximately 22 per cent. The use of water was nearly proportional to the leaf area and still more nearly proportional to the growth in length. The coefficient of correlation between the use of water and the leaf area for all the trees used (of which those given in table I are a part) is 0.97 ± 0.11 , and that between use of water and growth in length is 0.995 ± 0.002 . There is apparently no relation between soil-moisture content and either use of water or growth in length as here shown.

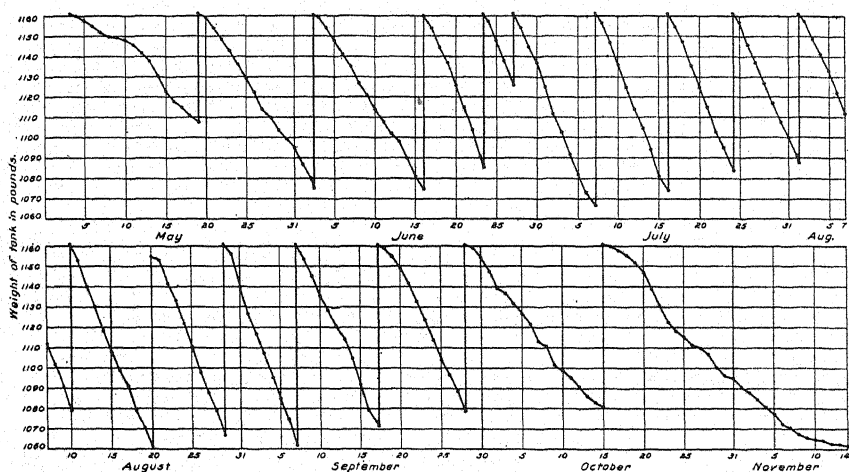


FIG. 3. Use of water by prune tree during the season of 1922, at Mountain View. The weight of the tank with soil containing 22 per cent. of moisture was 1161 pounds and the weight when the soil was at the wilting coefficient (11.9 per cent.) was 1070 pounds.

A further example to show that the rate of use of water by young prune trees in containers was not affected by the amount of water in the soil, provided the moisture content was not below the calculated wilting coefficient, is shown by the graph of figure 3, which presents data which were obtained from a tree on an automatically recording balance. The vertical portions show the applications of water, and the slope of each intervening portion of the graph indicates the rate of use of water, which was clearly no greater with high than with low soil-moisture contents.

When the soil-moisture content was reduced to a percentage corresponding approximately to the calculated wilting coefficient, the trees wilted and did not revive until water was applied to the soil. A remarkable degree of agreement between the observed and the calculated wilting coefficient is shown in table II in a number of cases, which are representative of a much larger number, for two different localities, and at different times during the growing season.

Still further evidence of the importance of the wilting coefficient as a critical point in the process of soil-moisture depletion by plant transpiration was secured from studies on the width of stomatal openings. Apricot, prune and peach trees growing on soil with water content higher than that corresponding to the wilting coefficient showed markedly wider stomatal openings by day than did those grown on soil with water content at or near the wilting coefficient. The average results obtained with prune trees during a 24-hour period beginning at 6 A. M., September 11, 1924, are shown

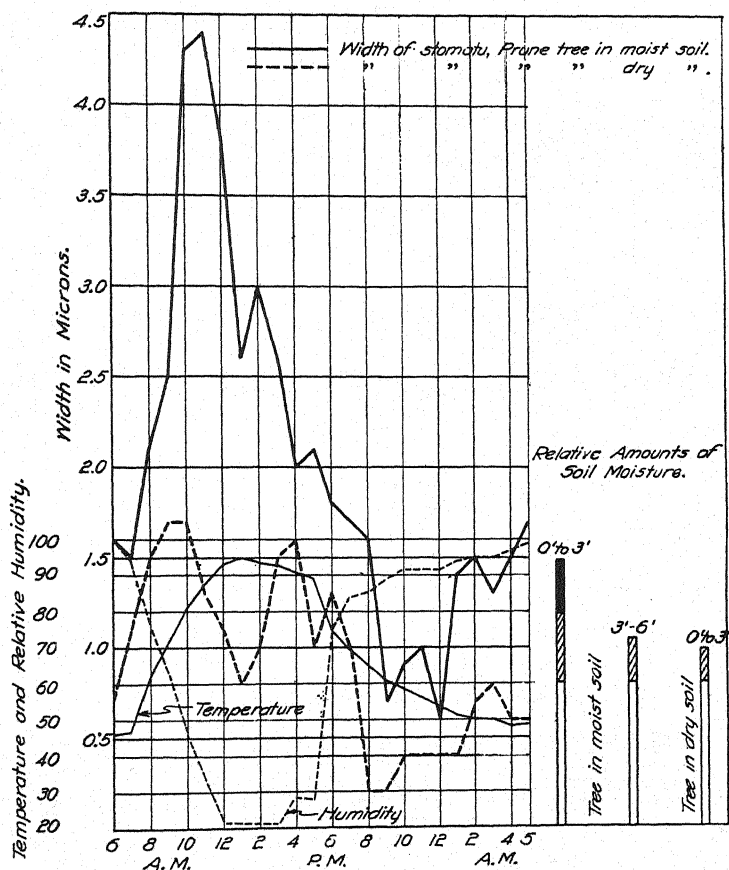


FIG. 4. Width of stomatal opening on prune trees in moist soil and in dry soil at Mountain View, California, September 11, 1924. Temperature and relative humidity are shown by the light lines in the lower left corner. Relative amount of soil moisture above the wilting coefficient is shown by the solid black column; relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion.

graphically in figure 4. The usual diurnal variation in stomatal widths is shown for both moist and dry soils, but the average maximum width for the moist soil is seen to have been about three times as great as for the dry soil. Similar results obtained with peach trees under climatic conditions essentially the same as those to which these prune trees were subjected are shown in figure 5, along with a similar graph for another prune tree on moist soil. However, differences in width of stomatal openings of leaves on any of the trees studied could not be detected when the soil-moisture contents varied but did not fall to the wilting coefficient. The average measurements of

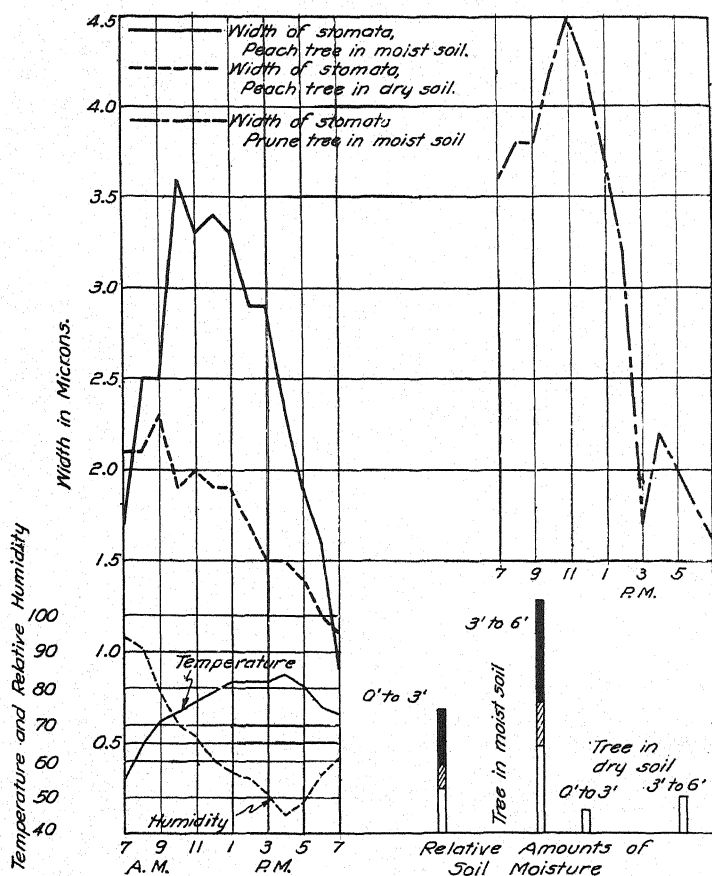


FIG. 5. Width of stomatal opening on peach and prune trees in moist soil and peach tree in dry soil at Delhi, October 1, 1924. Temperature and relative humidity are shown in lower left corner. Relative amount of soil moisture above wilting coefficient is shown by solid black column; relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion.

stomata of leaves on peach trees, one of which was on a soil with high moisture content, and the other on a soil with low moisture content but still above the wilting coefficient are shown graphically in figure 6. The graphs show that no real differences exist in width of stomatal opening. The stomata appear to have behaved as usual as long as the soil-moisture content was higher than the wilting coefficient. These results also substantiate the data given in table I, in that the trees were not affected until the soil moisture was reduced to the wilting coefficient.

TABLE II

RELATION OF THE OBSERVED TO THE CALCULATED WILTING COEFFICIENT FOR YOUNG PRUNE TREES IN CONTAINERS

TREE NUMBER	DATE WILTED	SOIL-MOISTURE CONTENT AT TIME OF WILTING	WILTING COEFFICIENT CALCULATED FROM MOISTURE EQUIVALENT
		per cent.	per cent.
3	May 5, 1922	11.6	11.1
3	August 14, 1922	10.5	11.1
3	September 28, 1922	11.2	11.1
5	August 25, 1922	13.0	12.7
5	October 7, 1922	12.7	12.7
5	May 3, 1923	12.3	12.7
6	August 21, 1922	11.6	11.9
6	September 2, 1922	11.7	11.9
6	April 29, 1922	11.3	11.9
19	June 13, 1924	11.1	11.2
20	June 12, 1924	11.0	11.4
10	July 7, 1924	11.9	11.6

Trials with mature peach and prune trees showed that growth in length of shoots could not be prolonged indefinitely by maintaining high soil-moisture contents throughout the season, and the same result was secured with young trees in containers. If the average percentage of water in leaves and bark and wood of all parts of the tree may be used as a criterion of maturity the results obtained with bearing peach trees indicate that trees growing on wet soils matured at the same time as trees on nearly dry soils for the mean water content of these parts of the tree was approximately the same in the autumn for both soils. Furthermore, young trees on moist soil held in containers dropped their leaves at the same time in the fall as those on drier soils. However, defoliation could be brought about during periods of high evaporating conditions by withholding water until the trees wilted.

The data secured in these studies seem to have an important bearing on a number of questions regarding the relation between irrigation and the hardening or maturing of the wood and buds of fruit trees. An abundant supply of soil moisture throughout the season can not alone account for the immaturity of the current growth of the tree and so-called winter injury that seems to be a result of such immaturity, at least under the conditions of these experiments.

The correlation between leaf area and the use of water by trees is illustrated in a concrete way by results obtained in one of the orchards at the Branch of the University of California at Davis. This orchard consisted

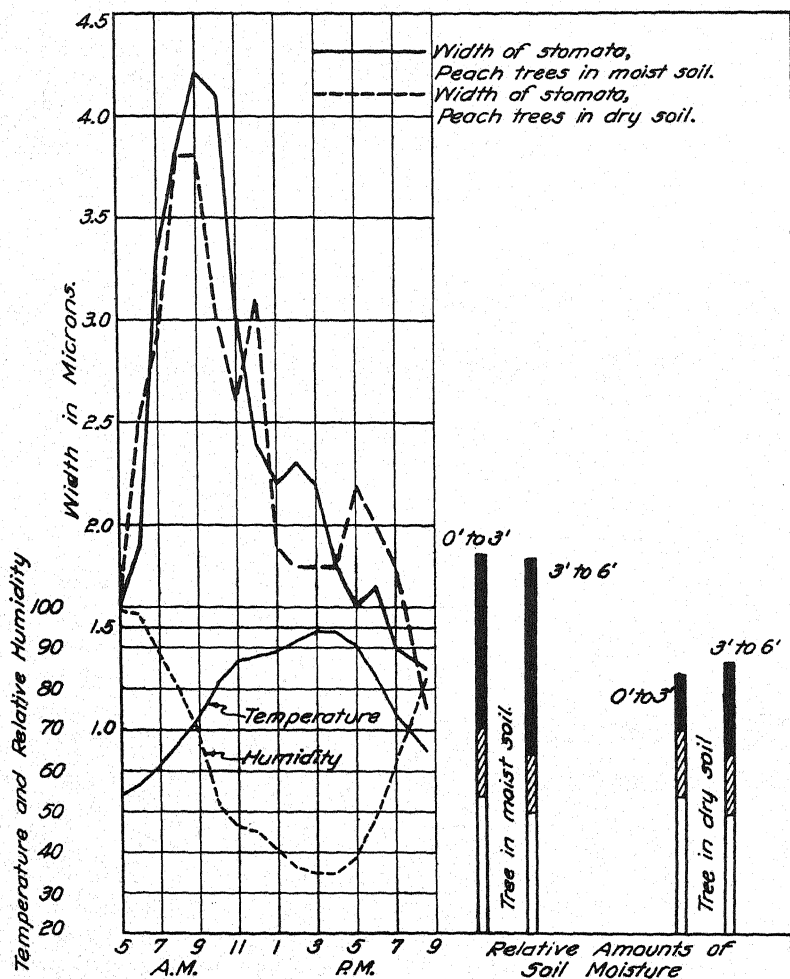


FIG. 6. Width of stomatal opening on peach trees on moist soil and on dry soil at Davis, California, July 9, 1925. Temperature and relative humidity are shown by light lines in lower left-hand corner. Relative amount of soil moisture above the wilting coefficient is shown by solid black column; relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion.

of suitable plots of cherries, plums, prunes, pears, peaches, and apricots, with different spacings. Thus, in the first block the trees were 12 feet apart, in the second, 16 feet apart, and so on until the trees of the last block were 36 feet apart. The trees were planted in 1915 and at the present time only those in the 30-foot and 36-foot blocks are alive. The trees in

the 12-foot block showed evidence of drought during the summer of the fourth season. After the fourth or fifth year growth was less and injury (such as sunburn) was greater in the more closely planted blocks than in the others. Those of the 12-foot block showed signs of drought, as evidenced by curling and dropping of the leaves, from two to five or six weeks before those of the 24-foot block showed these symptoms. The greater leaf area, in proportion to the moisture available to the trees, in the closely planted blocks was probably directly concerned with the premature decline of these trees.

The relation of leaf area to use of water by the tree has an important bearing on irrigation practice, especially when alfalfa is grown in the orchard as a continuous cover crop. The combination of trees and alfalfa requires more water than do trees alone. The writers are of the opinion that any benefits derived by the trees from the growing of alfalfa in this way are probably due to causes other than lessened transpiration, on the part of the tree, though this cause is often given to explain benefit to the trees apparently due to the cover crop.

The writers wish to express their thanks to Dr. B. E. LIVINGSTON, of Johns Hopkins University, who kindly read the paper and offered many helpful suggestions.

UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA

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DOES THE PEA PLANT FIX ATMOSPHERIC NITROGEN?

DEAN BURK

LIPMAN and TAYLOR (3) several years ago reported the fixation of atmospheric nitrogen by wheat and barley plants grown in culture solutions. Preliminary experiments with peas grown under similar conditions did not, however, show a similar power of fixation. These authors stated that their results with peas were inconclusive, since too small a number of plants were grown and statistical methods were not employed. They recommended that further studies be made. In their experiments only twenty one-liter jars, each containing three pea plants, were employed; no attempt was made to maintain sterile conditions; the culture solution used was tap water of unknown composition except for its nitrogen content, which was 1.0 mg. per jar; and since no statistical method was employed, the nitrogen contents of the seeds were not very accurately known. The following significant changes and improvements have been made in the experiments reported here. An adequate statistical method for the interpretation of analyses of seeds and harvested plants has been employed; sterile conditions have been maintained up until the moment of planting; two series were run, each differing with respect to the amounts of nitrogen supplied beyond that contained in the seed; the solutions were made up with distilled water and definite amounts of chemicals, and were analyzed for nitrogen both before and after the plants had grown in them; and at the end of the growing period the residual solutions were tested qualitatively for the traces of nitrogen contained in them.

Experimental

The general experimental procedure was much the same as that employed by LIPMAN and TAYLOR. The Dwarf variety of *Pisum sativum* was used. There were two plants in each jar, the jars having a capacity of two liters. The solutions of the two series, differing from each other only with respect to the amounts of nitrogen, and this in the form of nitrate, were as follows: The salts are expressed as mols, the water as liters, and the concentration as molality.

Series	KH_2PO_4	MgSO_4	CaSO_4	$\text{Ca}(\text{NO}_3)_2$	H_2O	Total concentration
1 No nitrate	0.412	0.364	0.120	none	up to 40	0.0224
2 Nitrate added	0.412	0.364	0.120	0.012	up to 40	0.0227

The salts were added from their molal solutions, except CaSO_4 , in which case 8.5 liters of a saturated solution were used. It was assumed that this

volume contained .120 mol. The solutions were analyzed for nitrogen by both the Devarda and modified Gunning-Kjeldahl methods (Davidson scrubbers were used), using three 200 cc. aliquots of each, and running four blanks on the chemicals with distilled water. The net totals of milligrams of nitrogen for each solution per jar were: Solution 1, 0.0 mg., solution 2, 8.0 mg., with an accuracy of ± 0.2 mg. Baker's analyzed chemicals without purification were employed. Two liters of solution were poured into each of the Mason jars, covered with a large amount of cotton, which was then tightly bound on, and sterilized for an hour in the autoclave at 15 pounds pressure. The jars had been previously cleaned twice with commercial concentrated hydrochloric acid and then rinsed thoroughly with distilled water. They were covered with paper on the outside. New two-hole corks which would fit tightly into the jars were paraffined and sterilized in the same manner as the jars. They were fitted into the jars at the moment of planting. The Dwarf pea seeds were selected to a size of 21 ± 1 centigrams and were sterilized as follows. They were placed in HgCl_2 (1/5000) for five minutes and then in sterilized distilled water for one hour and fifty-five minutes, a method suggested by Dr. LIPMAN. This operation was repeated twice. They were then placed as aseptically as possible in a large ten-inch sterilized Petri dish and incubated at 28 degrees for four days, during which time no visual trace of contamination by microorganisms developed. Sterile jars, solutions, corks, and seedlings were then taken to the greenhouse where the planting was done and where the plants remained throughout their entire growth period, which was 58 days from planting until harvest (September 27 to November 24). The peas were fitted into the holes in the corks with the assistance of sterilized cotton, which also served as a protection for the solution from the dust of the air. The only additional substance added to each of the jars was 5 cc. of a 0.5 per cent. solution of FeSO_4 , as follows: 1 cc. on the 18th day after planting, 2 cc. on the 25th day, and 2 cc. on the 30th day. No additional water was added. After harvesting, the plants of each jar were separately dried at 96°C . for 26 to 38 hours, weighed, and analyzed for total nitrogen by the modified Kjeldahl method. The digestion was carried on for a full three hours. An aliquot of 500 cc. of the total solution remaining in each of the jars was analyzed in a similar manner. As these solutions were found by qualitative tests to contain no nitrate, the Devarda method was not employed in their analysis. Dilute N/50 standard acid and alkali were used when analyzing the solutions; in all other cases, N/10 solutions. Hence the limits of accuracy, on a basis of 0.1 cc., are 0.03 and 0.1 mg. respectively. Sufficient reagents for all Kjeldahl analyses were prepared in one large lot. The nitrogen content of these reagents was determined by a sufficient number of blanks. At the commencement of the experiments the original lot of seeds

(21 ± 1 cg. each) was analyzed by the modified Kjeldahl method in 15 sets of 2 each. Also fifteen sets of two seedlings each were analyzed. These seedlings were sterile and were six days old, two days older than those which were planted. The purpose of analyzing the seedlings was to determine if any loss of nitrogen occurred during germination. In determining the statistical mean of the nitrogen contents of the seeds, the results for seeds and seedlings, after being calculated separately for purposes of comparison, were then calculated together, since by so doing the accuracy of the values of both the arithmetical mean and the probable error was increased.

The usual statistical methods have been employed. The probable error is taken to be the product of the constant, 0.6745, and the standard deviation, all divided by the square root of one less than the number of variants. The standard deviation is the square root of the quantity obtained by dividing the sum of the algebraic squares of the deviations from the arithmetical mean by the total number of variants. The probable error of the difference between two means is the square root of the sum of the squares of the probable errors of each mean. The "minimum difference required for significance" is calculated by multiplying the probable error of the difference of the means by the usual factor 3.2. This calculated minimum difference when compared with the actual difference obtained shows immediately the exact quantitative value of the experimental results.

The following observations were made during the growth period. The number of days refers to the number of days after planting. At 21 days, the lower portions of plants of series 1 were dying. At 25 days, the lower portions of all plants of series 1 were apparently dying or dead, and about one sixth of the plants of series 2 were similarly affected. At 26 days, one third of the plants of series 2 were affected. At 30 days, flowering had commenced, mostly in plants of series 2. At 39 days, the entire leafage of series 1 was practically dead, becoming rather suddenly so over a period of four days. The flowering plants were the most alive. Nearly all the green portions of series 2 were dying also. At 42 days, all plants of series 2 and one half those of series 1 were sending forth new shoots on the lower portions of the tops. At 51 days, increasing green growth in series 2, but in series 1 the new green parts were dying again. At 56 days, the plants of every jar in series 2, except three, were alive, green, and healthy; the plants of every jar in series 2, except two, were without green foliage of any sort. The solutions of all jars of both series when tested with the diphenylamine reagent showed that there was less than one part in ten million of nitrogen as nitrates; and as the tests were all negative, there presumably were no nitrates. As this reagent detects various forms of oxygen-nitrogen compounds such as nitrites, nitro compounds, and others, it may demonstrate their absence also. Tested for ammonia by Nessler's reagent (and therefore

for many other amines also) all solutions, regardless of series, which contain living plants gave absolutely negative tests; and all solutions, regardless of series, in which the tops have died, showed the presence of small amounts of ammonia. This amount however, when the volume of solution is taken into consideration, was 1 to 2 mg. per jar, and is therefore of significant proportions. The roots of the plants gave no evidence of nodules. There had been no aphids on any of the plants. Only a few of the plants had mold. In general, the ratio of root to top was approximately constant, that is, the larger the top, the larger the root. At 58 days, plants were harvested and every root examined for nodules. None were found. The majority of plants had flowered; sixteen had produced pods containing seeds; the water transpired had been small, about 800 cc. per jar in series 1 (quite uniformly) and 800 cc. to 1,000 cc. in series 2. In none of the plants had there been any mortality during the first three weeks of growth.

The analyses for the nitrogen-free solutions, and plants grown in them, are given in table I. Those for solutions containing nitrogen are shown in table II. The nitrogen content of seeds and seedlings six days old is given in table III, and the results are summarized statistically in table IV.

Discussion

Within each series, the dry weights of the plants were quite closely proportional to the nitrogen contents, in accordance with the law of the minimum. Under these conditions of nitrogen starvation the pea plant showed marked avidity for ammonia and nitrate in the culture solution.

The fact that, in LIPMAN and TAYLOR's experiments most of the plants lived, while in series 1 of these experiments most of the plants died, might possibly be explained either by the difference of the critical 1 mg. of nitrogen in the culture solution or by traces of an essential element which the tap water may have possessed. The death of plants of series 1, from all outward appearances, was due to physiological rather than to parasitic causes.

The presence of excreted nitrogen in the culture solutions, together with the very probable and generally accepted fact that plants may lose nitrogen gas during photosynthesis (early shown by the researches of SIR HUMPHREY DAVY, DE SAUSSURE, RIGG (4), DAUBENY (1), and DRAPER (2), make it possible that the pea plants in these experiments lost enough nitrogen to hide any evidence of fixation. See also SPOEHR (5) for a recent discussion of nitrogen loss during photosynthesis. Such a definite explanation has often been overlooked in the reports of the few experiments which have been performed along lines similar to these.

These experiments are an admirable inferential check on the positive experiments of LIPMAN and TAYLOR with wheat and barley. They indicate, first, the probable absence of bacterial intervention, and second, the general

TABLE I
SERIES 1. NITROGEN FREE SOLUTION

JAR NUMBER	DRY WEIGHT	N IN RESIDUAL SOLUTION	TOTAL N IN PLANTS AND SOLUTION	DEVIATION FROM MEAN
	cg.	mg.	mg.	
1	50	1.05	15.54	0.42
2	43	1.77	15.54	0.42
3	36	1.17	16.10	0.98
4	55	1.17	14.14	-0.98
5	47	1.05	14.84	-0.28
6	47	1.17	14.14	-0.98
7	47	1.50	lost
8	55	1.71	16.52	1.40
9	53	1.05	13.72	-1.40
10	43	1.65	15.40	0.28
11	47	1.98	17.78	2.66
12	40	1.05	13.44	-1.68
13	80	0.66	19.46	4.36
14	46	1.11	16.24	1.12
15	43	1.50	14.70	-0.42
16	50	0.78	14.66	-0.46
17	56	1.05	16.52	1.40
18	36	1.17	13.30	-1.82
19	41	0.66	14.52	-0.60
20	47	1.65	15.40	0.28
21	25	1.05	10.36	-4.76
22	46	0.99	14.14	-0.98
23	52	0.84	14.84	-0.28
24	40	1.98	13.16	-1.96
25	50	1.44	16.48	1.36
26	40	1.44	13.96	-1.16
27	34	1.32	12.18	-2.94
28	47	1.05	16.24	1.12
29	40	0.78	15.96	0.84
30	58	0.60	19.18	4.06
—	—	—	—	—
Mean	46.4	1.21	15.12	
Probable error	1.2		0.24	

Volume of residual solution, 1200 ± 50 cc. in every case.

Only two jars where plants had green tops, 13, 19.

Only two jars with no ammonia in solution, 13, 19.

Plants larger than others, 4, 6, 8, 9, 13, 22, 30.

Pods on, 13.

TABLE II

SERIES 2. SOLUTION WITH NITRATE, 8 MG. N PER JAR IN SOLUTION

JAR NUMBER	DRY WEIGHT	N IN RESIDUAL SOLUTION	TOTAL N IN PLANTS AND SOLUTION	DEVIATION FROM MEAN
	Cg.	mg.	mg.	
31	82	0.21	21.14	-0.41
32	60	0.66	lost	
33	59	0.06	21.56	0.01
34	68	0.00	21.56	0.01
35	38	0.00	14.56	-6.99
36	73	0.06	22.40	0.85
37	87	0.06	22.96	1.41
38	72	0.36	18.34	-3.21
39	72	1.08	20.58	-0.97
40	72	0.30	21.98	0.43
41	60	0.63	21.84	0.29
42	72	0.42	20.82	-0.73
43	69	0.00	21.98	0.43
44	69	0.30	19.46	-2.09
45	75	0.36	20.82	-0.73
46	84	0.06	21.98	0.43
47	71	0.06	21.14	-0.41
48	90	0.21	21.00	-0.55
49	66	0.06	20.40	-1.15
50	70	0.06	17.36	-4.19
51	104	0.21	20.82	-0.73
52	75	0.03	20.82	-0.73
53	97	0.03	24.36	2.89
54	102	0.78	24.08	2.53
55	78	1.08	23.94	2.39
56	91	0.72	24.22	2.67
57	92	1.08	22.54	1.99
58	95	0.45	23.94	2.39
59	75	0.06	27.16	5.61
60	83	0.06	22.68	1.13
—	—	—	—	—
Mean	73.4	0.31	21.55	
Probable error	1.8		0.30	

Volume of residual solution (± 50 cc.) 1200 cc., 32, 33, 35, 39; 1000 cc., 54; 1100 cc., the remaining jars.

Only jars without green tops, 35, 39, 50.

Only jars with ammonia in solution, 35, 39, 50.

Plants smaller than others, 35.

Pods on, 32, 40, 41, 43 (two), 44, 47, 48, 51, 53, 54, 57, 58, 59.

TABLE III
NITROGEN CONTENT OF SEEDS, AND SEEDLINGS SIX DAYS OLD

SEEDS			SEEDLINGS		
SET NUMBER	N	DEVIATION FROM MEAN	SET NUMBER	N	DEVIATION FROM MEAN
	mg.			mg.	
1	16.24	-0.43	1	15.96	-0.47
2	18.48	1.81	2	16.10	-0.33
3	15.82	-0.85	3	16.38	-0.05
4	16.80	0.13	4	15.26	-1.17
5	17.22	0.55	5	15.68	-0.75
6	16.82	0.13	6	16.66	0.23
7	15.96	-0.71	7	17.50	1.07
8	14.70	-1.93	8	15.26	-1.17
9	18.62	1.95	9	19.60	3.17
10	18.62	1.95	10	19.20	1.77
11	16.84	0.17	11	14.70	-1.73
12	15.54	-1.13	12	17.78	1.36
13	18.86	2.19	13	13.86	-2.57
14	14.66	-2.01	14	17.54	1.11
15	15.82	-0.85	15	15.82	-0.61
—	—	—	—	—	—
Mean	16.67			16.43	
Probable error	.242			.257	

Seeds and seedlings together: Mean 16.55; probable error 0.19.

TABLE IV
STATISTICAL SUMMARY OF RESULTS

DETERMINATIONS	SERIES 1		SERIES 2	
	N	DRY WEIGHT	N	DRY WEIGHT
	mg.	Cg.	mg.	Cg.
Original solution0		8.0	
Original seed	16.55 ± 0.19	42.	16.55 ± 0.19	42.
Total initial	16.55 ± 0.19	42.	24.55 ± 0.19	42.
Total final	15.12 ± 0.24	46.4 ± 1.2	21.55 ± 0.30	73.4 ± 1.8
Probable error of dif- ference of means	± 0.30		± 0.36	
Loss during growth period	1.43 ± 0.30		3.00 ± 0.36	
Gain during growth period		4.4 ± 1.2		31.4 ± 1.8
Minimum difference re- quired for signifi- cance	0.96		1.15	
Per cent. gain or loss...	-9.6	+10.5	-12.2	+74.8

sufficiency of their technique, in a manner, it may be added, which would be hard to equal.

Summary

The Dwarf variety of *Pisum sativum* when grown under the general conditions stated, either in the absence or in the presence of additional nitrogen in the original culture solution, and with sterile conditions maintained up until the moment of planting, has shown upon statistical treatment of the experimental results a small unqualifiable *loss of nitrogen*. This nitrogen was not lost during germination, but afterwards, during the growing period of the plant.

The writer wishes to express appreciation of suggestions offered by Dr. CHARLES B. LIPMAN.

DIVISION OF PLANT NUTRITION,
UNIVERSITY OF CALIFORNIA.

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THE DETERMINATION OF POLYSACCHARIDES¹

Because of the complexity of the polysaccharides, our knowledge of them is much less definite than our knowledge of the simple sugars, and this is strikingly reflected in the relative indefiniteness of the methods for determining them. In most cases, they need to be hydrolyzed to simpler substances as a preliminary step. Since the hydrogen ion is the universal catalyst for these hydrolyses, and since in most cases several polysaccharides occur concomitantly, the decided lack of specificity of determinations is evident. The most hopeful direction of improvement is in the development of biochemical methods, involving the use of specific micro-organisms and enzymes.

In the following discussion it has been the aim of the committee to indicate the methods which are available for determining a given substance, and, where possible, to suggest the preferred one. Working directions are not given since the original citations are readily available, and since in many cases the committee's choice of a method may not be the one adopted by the investigator.

Cellulose

The determination of true cellulose is a laborious procedure, and has been applied almost exclusively to the analysis of wood and paper. The one usually used is the chlorination method of CROSS and BEVAN, as perfected by SCHORGER (19). Its success depends largely upon the proper grinding of the material. Twigs have been ground very handily in a pencil sharpener (11).

The old method for crude fiber, used in the proximate analysis of feeds, is at times useful in physiological investigations (28). It gives a rough idea of the cellulose of the tissues, and is much more easily carried out than the method for true cellulose.

Starch

Preliminary treatment.—It is the consensus of opinion among biological chemists that a starch determination must be preceded by an extraction of the material with dilute alcohol to remove the simpler carbohydrates. This holds not only in materials which are obviously relatively high in sugar and dextrans and low in starch, such as succulent tissues, but also in such materials as cereal grains, where the sugars are present in very small quantities (14). The concentration of alcohol and the details of procedure differ

¹ Separates of the various sections of the report of the Committee on Methods of Chemical Analysis of Plant Tissues can be purchased at nominal cost from the chairman of the committee, Dr. W. E. TOTTINGHAM, Agricultural Chemistry Building, University of Wisconsin, Madison, Wisconsin.

with the material involved,² but use of alcohol much more concentrated than 50 per cent. is liable to precipitate dextrans.

Gelatinization.—The residue from the alcohol extraction is freed from all but traces of alcohol, and to it is added sufficient water to make a thin suspension. The concentration of the suspension will depend on the starch content, and will be varied so as to allow for proper aliquoting for subsequent analysis. Boiling at ordinary pressure is used, and in general is to be recommended over autoclaving. If the material is ground rather fine, the latter treatment is unnecessary.

Saccharification.—Diastase in one form or another is very much to be preferred over acid for converting the starch to sugar, because of the more specific action of the enzyme. Takadiastase has been successfully used by several careful investigators (6, 22). Although HORTON (12) questions its reliability in some cases, it is generally conceded to be a trustworthy and convenient hydrolysant for analytical work. Salivary diastase was used by TOTTINGHAM and GERHARDT (23). The most commonly used source of diastase is barley malt. The principal precautions in its use are to obtain very active malt, to grind it fresh before extraction, to make careful blank determinations, and to test the solution for the absence of starch and dextrin.

When the analyst is satisfied that starch is practically the only polysaccharide in his material which is hydrolyzable by dilute hydrochloric acid, or when for other reasons he wishes to include some hemicelluloses in his hydrolysis, the diastase treatment may be replaced by heating the material at about 100° C. with about 2 per cent. hydrochloric acid, for from 1 to 3 hours. The strength of acid used has varied with different workers, but since the process is empirical the above average strength may be accepted as suitable in most cases. The time of heating employed has varied with the nature of the material; since this is also empirical it is either decided upon arbitrarily by the analyst, or, by means of trial hydrolyses, he determines the proper time for his particular material.

After saccharification of the starch it is customary to boil and filter the material, and then perform an acid hydrolysis on the filtrate. This is because all preparations of diastase contain some maltase; hence the hydrolysate contains a mixture of maltose and glucose, and a simple determination of reducing sugars will not suffice. DAVIS and DAISH (6) solved for the two sugars by means of a simultaneous equation. It is simpler, however, to remove interfering polysaccharides by filtration, or by alcoholic precipitation (25) and filtration, and then to hydrolyze the filtrate by boiling with approximately 2 per cent. hydrochloric acid for 2.5 hours, and determine the resultant sugar as glucose.

² See previous report of this committee, *Plant Physiol.* 1: 397-402. 1926.

Results should be reported in terms indicative of the method used; that is, as starch if diastase is used, otherwise as acid hydrolyzable material including starch.

*Clarification.*³—It has usually been considered necessary to clarify all sugar solutions before analysis by either polariscopic or chemical means. MORRIS and WELTON (15) question the necessity of clarifying all materials, as do WILLAMAN and DAVISON (27) in using the picrate method. The safest procedure is for the analyst to determine for his own material whether clarification is required. Tungstic acid and phosphotungstic acid (18) have been used to clarify sugar solutions, but neutral lead acetate is still the commonest reagent for this purpose. Deleading should be brought about with di-sodium phosphate, as it is least likely to cause the occlusion of sugars by the precipitate (9).

Determination of reducing sugars.—The analysis of the final hydrolysate can be carried out by any of the methods for reducing sugars given in a subsequent section of the report of this committee.

Hemicelluloses

It is regrettable that this ill-defined group of carbohydrates is still in a very unsatisfactory state as regards methods for its dissection and analysis. The group is roughly defined as including insoluble polysaccharides and related compounds which are hydrolyzed by boiling with dilute mineral acids; in other words, it contains pentosans, pectins, galactan and mannan. Some of these can be determined separately. The latter is greatly to be desired, and, indeed, its importance cannot be over-emphasized. The usual reason for embodying a number of these substances in the same analysis is that they represent reserve food materials in plant tissue. This, however, is largely an assumption, since we have only circumstantial evidence that this is the case. In any event, such group determinations give little indication of changes in proportion of constituents.

The group reagent for hydrolyzing the hemicelluloses differs with different investigators. Usually it is a dilute hydrochloric acid solution, such as 3 per cent. (24), 1 per cent. (21), and 0.5 per cent. (22). With some tissues, however, sulphuric acid seems to be preferable (23). The length of time of hydrolysis varies, but is usually from 1 to 4 hours. The conclusion seems to be warranted from the data at hand that each investigator should make a preliminary study of his material to ascertain what acid, what strength of acid, and how long a time of boiling are necessary to give the maximum amount of reducing sugars without appreciably affecting the true cellulose. Since the method is empirical, the following values are recommended either for direct use or as a basis for a series of trials: 2 per

³ For details see report of this committee on determination of simple sugars.

cent. hydrochloric acid for 2.5 hours, at 100° C. These unknown factors and uncertainties emphasize the desirability of separating the individuals of this group, instead of employing a blanket method. After hydrolysis the solution is neutralized, clarified, and the reducing sugars determined by any method preferred.

Some progress is being made in the biological analysis of tissues, whereby specific substances are removed by enzymes or by micro-organisms. This appears to be a fruitful field for investigation, but no recommendations are as yet warranted.

Pentosans

There are two general methods for analyzing for the pentosans. One is the old "official" method of converting the pentosans to furfural and determining the latter in the distillate. The other is their hydrolysis to pentoses and the determination of the latter by copper reduction, after fermenting away the coincident hexoses. The first, or phloroglucinol method, is admittedly subject to many errors; but the only improvement on it, the method of PERVIER and GORTNER (17), is rather cumbersome to carry out. The fermentation method has been apparently successful in the hands of several investigators (5, 21, 23). This method is based upon the assumption that pentoses are not destroyed by yeast. Several strains of pure yeast have been found to attack the pentoses after fermentation of the hexoses, and hence the method is reliable only when the action of the yeast is stopped as soon as the fermentation of hexoses is complete.⁴ Mrs. ABBOTT (1) has made an extensive study of the method and has found many pitfalls which must be avoided. The most important precautions are to use pure cultures of yeast, to adjust the medium to the proper acidity, and to test aliquots from time to time for the completion of the fermentation. Pure cultures can be secured from American Type Culture Collection, 637 South Wood Street, Chicago.

Galactan and mannan

These substances have not received much attention from the standpoint of analytical methods. Galactan is usually oxidized to mucic acid by means of nitric acid. Mannan is converted to mannose, and the latter quantitatively to its hydrazone. The reader is referred to the work of DORE (7) and of SCHORGER (20), respectively, for details of those methods.

Inulin

Inulin and the inulides are hydrolyzed to fructose, and the latter determined polarimetrically or by reduction (13).

⁴ Private communication by C. O. APPLEMAN.

Pectins

The determination of the pectic substances is in an unsatisfactory condition, probably due to the fact that we have so little definite information about their composition. There was a time when one was limited to precipitating the pectin from solution by adding two volumes of alcohol, and weighing the precipitate. The latter contained protein, enzymes, and gums as well as pectin, hence the method was quite unsuitable. Then as more knowledge of the composition of the pectins was gained, other more specific methods were proposed.

VON FELLEBERG (10) proved that methyl alcohol is a constituent of all pectins; and as he believed it occurred in a constant ratio, he proposed to determine pectin by analyzing for methoxyl. It has been shown, however, that pectins from various sources differ in their methoxyl content, and hence this method is invalid. WICHMANN (26) proposed to saponify the pectin with alkali, precipitate the pectic acid with HCl, boil with the latter, and weigh the precipitated pectic acid. The objection to this method is the fact that furfural is always obtained by the action of HCl, and this represents a disintegration of the pectin molecule. CARRÉ and HAYNES (3) made exhaustive extractions of the finely ground tissue, usually as many as sixty extractions being required. The pectin thus dissolved was saponified, forming sodium pectate. The pectic acid was freed by acetic acid, and precipitated as the calcium salt. The latter was weighed as such. Besides being extremely laborious, this method is open to the criticism that the calcium pectate is probably not uniform in composition and that it occludes other substances. AHMANN and HOOKER (2) took advantage of the facts that soluble pectin has several carboxyl groups, either free or esterified, and that when pectin is boiled with excess of alkali a quantity of the latter will be absorbed which is proportional to the amount of pectin present. Their method is direct and simple, and gave results on pectin preparations comparable to those by the CARRÉ-HAYNES method. DORE (8), and NANJ, PATON and LING (16) boiled pectin with HCl so as to remove CO₂ from the carboxyl group of the galacturonic acid units of the pectin. The CO₂ was determined by weight or by titration. Although this was found to be an accurate method for galacturonic acid, it was not for pectin, probably because of a varying content of this substance in the pectin. Finally, CONRAD (4) has developed a way of extracting the insoluble pectic compounds of a tissue with ammonium citrate and with very dilute HCl, and determining the resultant pectic acid by the method of CARRÉ and HAYNES.

It is impossible for the present committee to make a definite recommendation. The AHMANN-HOOKER method appeals because of its simplicity. It is probably more important at present to investigate the pectin molecule and to devise methods than it is to try to analyze tissues quantitatively for pectins.

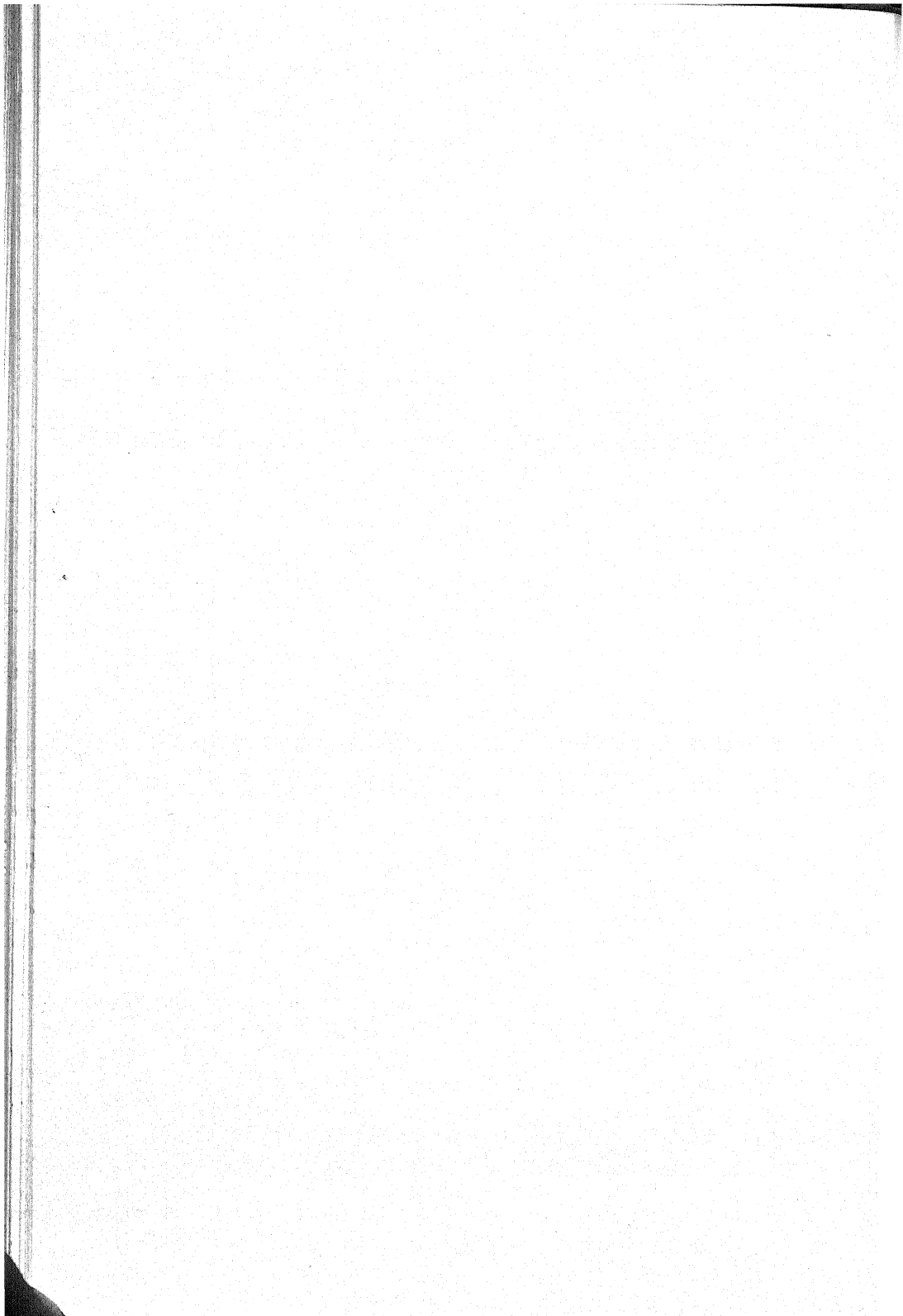
This report was organized by J. J. WILLAMAN for the Committee.

C. O. APPLEMAN,
W. E. LOOMIS,
T. G. PHILLIPS,
W. E. TOTTINGHAM (chairman),
J. J. WILLAMAN.

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BRIEF PAPERS

APPARATUS FOR CONTINUOUS DIALYSIS AT LOW TEMPERATURE

(WITH ONE FIGURE)

The authors have had occasion to carry out on a considerable scale the dialysis of plant tissue fluids, and have found the apparatus here described very convenient and effective. It can be used at any temperature, but in our work has been used in conjunction with a cold chamber to check enzyme action in the juice.

The supply of distilled water is provided by an ordinary still kept running during dialysis. The water enters at *W* (fig. 1), dropping through a layer of toluene *T* floated on the surface of the water in *R*, a carboy. *R* and *R'* act as reservoirs. The dialyzing system is enclosed in *F*, a "frigidaire" box in which the cold temperature is automatically held within fairly close limits. The water is cooled by passing through *C*, which may consist of a coil of block tin tubing, or a number of bottles in series. The bottle *B* is provided with a siphon adjustable for height at *A*. This siphon is of relatively small bore and requires 3 or 4 minutes to empty the contents of *B* into the diffusion vessel *V*. The latter has a large-bore siphon, which empties the contents of *V* into the drain *D* in less than one minute. The inner end of the large siphon drops into a small sump in the bottom of *V*, thus insuring the complete emptying of this vessel.

The diffusion vessel *V* is filled with water to the bend in its siphon. When *B* begins to discharge into *V*, the large siphon of the latter vessel is automatically brought into operation. The quick draining of *V* breaks the action of its siphon a few moments later, allowing the vessel to be refilled at once by the remaining discharge from *B*. The frequency of water changes in the diffusion vessel is regulated by the stopcock *S*, and also by drawing out to a small bore the end of the supply tube entering *B*. After the system has been adjusted, we have found that it will continue without further attention, changing the water once every hour for several days.

The size of the different containers may be varied to suit conditions. In our work the diffusion vessel accommodates twelve dialyzing tubes, each of 100 cc. capacity. Any change in the number or volume of the tubes used would of course necessitate a change in the adjustment of the upper siphon at *A*. In practice we have found it more convenient to keep the number of tubes constant, simply filling with water those not required in any particular experiment.—R. NEWTON and W. M. MARTIN, *University of Alberta*

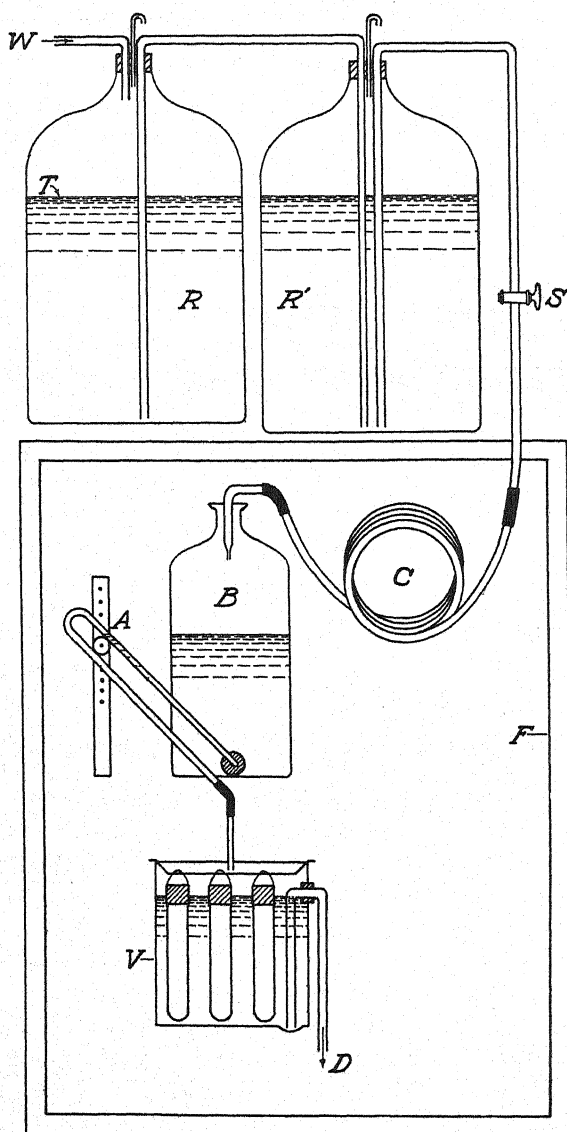


FIG. 1. Apparatus for continuous dialysis at low temperature.

A LABORATORY METHOD OF PREPARING STARCH FROM MAIZE SEED¹

Preparations of maize starch of a high degree of purity have been obtained from the seed by adapting the essential processes involved in the commercial manufacture of this product to laboratory conditions. The method may be extended to other grains by modification of certain steps in accordance with the nature of the fatty and proteinaceous substances present.

Clean maize seed placed in battery jars is steeped in a solution of sulphurous acid of a concentration of 1° B. (1.007 sp. gr.) for 24 hours at a temperature of 40° C. At higher temperatures, not to exceed 60° C., the time may be shortened. During the period of steeping, the strength of the solution is kept at approximately 1° B. by addition of further sulphurous acid from a stock solution. The sulphurous acid affords antiseptic conditions, has a pronounced bleaching effect, and so softens the grain that the starch is readily separated from the materials in which it is embedded.

The softened grain is then washed with water and ground in a meat grinder fitted with medium plates. Binding in the machine may be prevented by the addition of a little water from time to time. By kneading the wet pulp in a double layer of cheesecloth the starch fraction is worked through, leaving most of the embryo and pericarp tissue behind. A considerable portion of the remaining non-starch particles is removed by washing with water on a metal sieve having 100–120 meshes per inch. The crude starch is allowed to settle and the supernatant liquid is siphoned off.

Purification is effected by treatment with alkali. Enough water is added to the crude starch to make a flowing paste. This paste is poured slowly, with constant stirring, into about 5 volumes of a solution of NaOH not exceeding 0.45 per cent. in strength. A concentration of 0.70 per cent. NaOH gelatinizes starch very quickly and makes the separation of impurities very difficult or impossible. The mixture should be well stirred for half an hour, and further agitated at intervals over a period of 3 or 4 hours. The starch is allowed to settle, and the supernatant liquid containing the major portion of the impurities soluble in dilute alkali is siphoned off. The solid residue is washed with water and again allowed to settle.

The partially purified starch is taken up with distilled water and stirred thoroughly. If allowed to settle for a period of 10 to 15 minutes the coagu-

¹ Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 69. Published with the approval of the Director of the Station.

lated alkali-insoluble proteins fall to the bottom and the supernatant liquid, containing the starch in suspension, is siphoned off. This process is repeated three times. Alkali-soluble impurities still present, and the NaOH, remaining, may be removed by again suspending the starch in water and allowing it to settle 3 or 4 times over a period of two days. The last wash water should be clear and give a P_H of 6 to 7.

Finally the starch is placed over filter paper in a Büchner funnel and treated with 95 per cent. alcohol followed by successive portions of absolute alcohol to dehydrate. Fatty substances still present are removed by washing the water-free starch with ether. The starch is dried in crystallizing dishes at approximately 40° C.

Analysis of a sample of starch prepared by this method from common maize showed 0.035 per cent. nitrogen. A lot of waxy maize starch, which stains reddish-brown with iodine, prepared side by side with the above sample contained 0.013 per cent. nitrogen.—R. A. BRINK and F. A. ABEGG, *University of Wisconsin, Madison, Wisconsin.*

THE ACTION OF ETHYLENE IN ACCELERATING THE BLANCHING OF CELERY

Preliminary experiments to date have been concerned with the following points:

1. The effects of various concentrations of ethylene on the rate of blanching, on the crispness and flavor, and on the storing quality of the celery.
2. The effect of the treatments on the acidity of the celery juice, as an indicator of physiological changes in the plants.
3. The action of ethylene on the rate of respiration of the celery.
4. The effect of removal of carbon dioxide, a product of respiration, on the blanching process, and also the effect of excess amounts of carbon dioxide, as an index of the rôle of by-products of respiration.

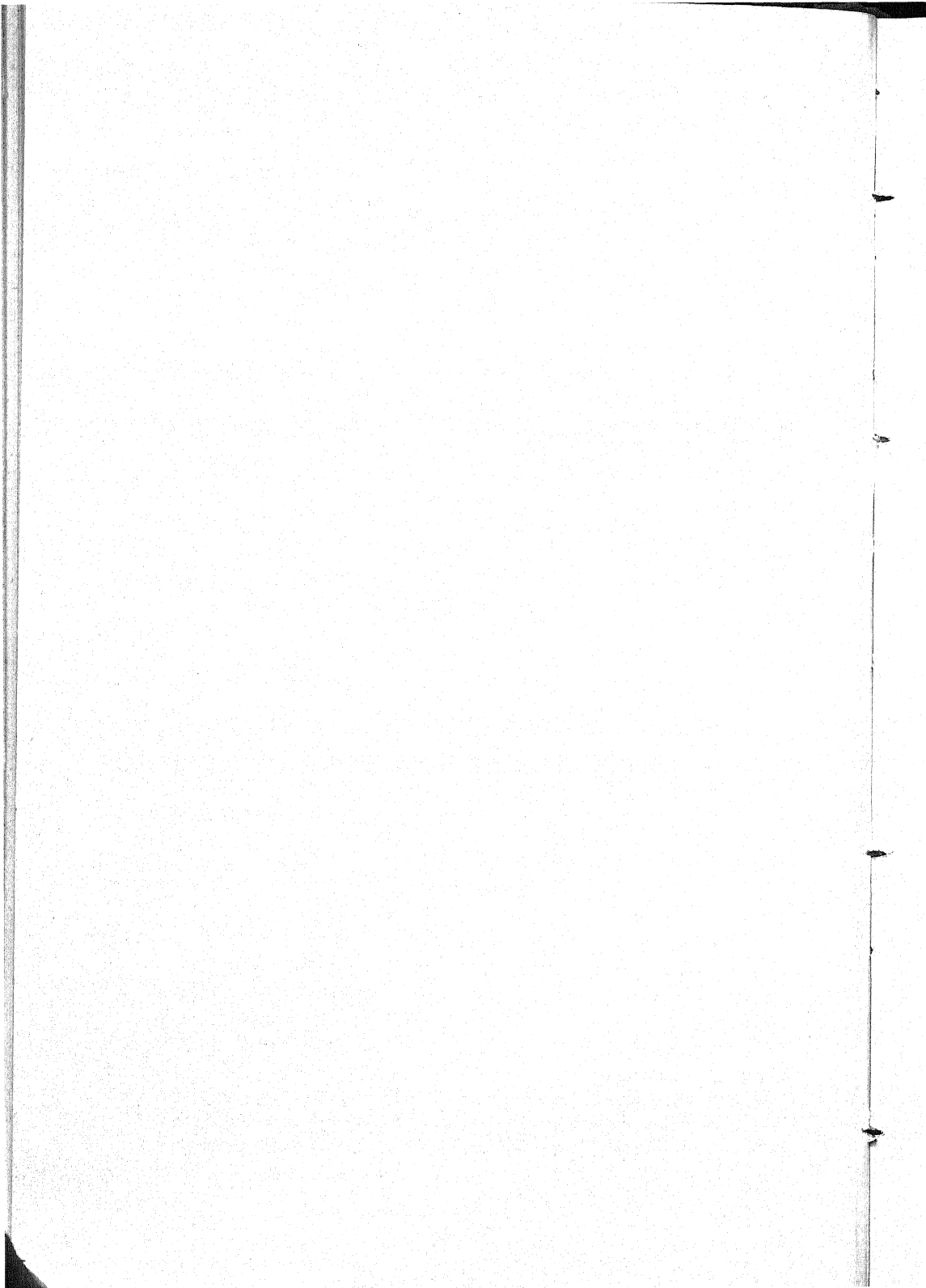
Concentrations varying from one part of ethylene in 500 of air, to one part in 50,000 of air, have been used. The most rapid and complete blanching took place where the lower concentrations were used. Very little difference was apparent between 1:25,000 and 1:50,000; these concentrations, however, were much superior to the stronger ones (1:2500 to 1:500), which showed little more blanching than the untreated check. The larger amounts of ethylene, particularly the concentration of 1:1,000, produced an injury characterized by splitting of the stalks on the inner surface, and pronounced pithiness of the stalks thus split.

The acidity of the celery juice was apparently not changed by the ethylene treatment. Differences in flavor were evidently not caused by the accumulation within the plants of by-products of respiration.

The rate of respiration was more than doubled by exposure to ethylene, as compared to the rate for untreated celery. The most rapid respiration, as indicated by the amount of carbon dioxide given off, took place in the sample exposed to one part of ethylene in 50,000 of air. In other words, the most rapid respiration was accompanied by the best blanching. Samples exposed to this concentration of ethylene were the most crisp and tender, but were the most subject to rot.

The removal of carbon dioxide by the use of calcium oxide and potassium hydroxide increased the rate of blanching and also the amount of rot. In the presence of very high concentrations of carbon dioxide, ethylene at the rate of 1:10,000 failed to produce any blanching at all.

These facts indicate that ethylene accelerates the blanching process by stimulating the activity of enzymes normally concerned with the breaking down of various compounds in the plant. With this in view, further studies are being initiated on the effect of ethylene on the enzymes of the celery plant, particularly oxidase, peroxidase, catalase, protease, cellulase, and pectase.—W. B. MACK, *Division of Vegetable Gardening, Department of Horticulture, State College, Pa.*



NOTES

The Philadelphia Meeting.—The third annual meeting of the American Society of Plant Physiologists was held at Philadelphia, December 28–31, 1926. The various sessions were held in the Veterinary Laboratories of the University of Pennsylvania, and in various other buildings in connection with joint meetings with Ecologists, Horticulturists, and the Physiological Section of the Botanical Society of America. The meetings were well attended, even though the meeting room was located inconveniently for those who desired to migrate from one meeting to another. The meeting Tuesday morning, December 28, was one of the finest ever held by any biological group, and set a standard that will be difficult to excel. The program committee deserves high praise for its work in arranging the invitation papers and others in groups somewhat related as to subject matter. The only regrettable feature was the fact that two groups of plant physiologists were meeting simultaneously, so that no one could hear all the papers. If all such meetings could be made joint meetings, held in a single room, so that anyone could hear all the papers if he so desired, it would be a good step forward.

Summer Meeting.—For two years the Corn Belt Section of the American Society of Agronomy and the Physiologists have held joint meetings in July. It had been proposed to hold a meeting at Purdue University this summer, but in view of the fact that the Agronomists will probably hold a meeting there within a year or two, the executive committee decided that it would be better to hold no summer meeting in 1927. This leaves us free to concentrate upon the next annual meeting, to be held at Nashville, Tennessee, in December, 1927. Every member should plan to attend the Nashville meeting.

Life Membership Award.—The first award of the CHARLES REID BARNES Life Membership was made at the banquet of the American Society of Plant Physiologists on the evening of Wednesday, December 29, 1926. The Society, at the suggestion of the committee charged with making the selection, has conferred the honor of life membership in the Society, *honoris causa*, upon Dr. BURTON EDWARD LIVINGSTON, Professor of Plant Physiology at the Johns Hopkins University since 1909, and Permanent Secretary of the American Association for the Advancement of Science since 1920. A full account of the award has been published in *Science*. Each year a new link in this living memorial to Dr. CHARLES REID BARNES will be added, and the banquet should prove a happy and appropriate time for making the announcement of the award.

Errata.—An occasional error creeps into every publication. The ideal of the Editorial Committee of this journal is to produce a publication as nearly free from mistakes of any kind as is humanly possible. We call attention to the small list of *errata* listed in connection with the table of contents of Volume I. Those who possess the journal should make the corrections at the place where they occur. We invite all of our readers and contributors to call to the attention of the editors any error found in PLANT PHYSIOLOGY, in order that proper corrections may be made.

Condition of the Society.—The growth of the American Society of Plant Physiologists has been truly remarkable during the past year, and the new year starts under the most favorable auspices. The membership numbers nearly 250, and the library subscriptions exceed 100 at the beginning of the second year of publication. The income of the Society during 1926 was more than sufficient for current needs, and we have a small surplus of Volume I, which will bring some hundreds of dollars into the treasury when completely sold out. Few scientific organizations have ever had such a promising start, in connection with the publication of a high class journal.

Back Numbers.—Institutions, libraries, and individuals who may desire to own a complete file of PLANT PHYSIOLOGY should take steps at once to acquire the first volume. The conservative policy of the Executive Committee and the Editorial Committee led to the publication of only a small number of copies of Volume I, a strictly limited edition. The surplus left is not nearly large enough to give each American plant physiologist the opportunity to own one. The large demand for it was not anticipated, and as a result there is imminent danger of exhaustion of the first volume. Those who are interested in Plant Physiology should not wait to be invited to join the Society, but should write to the Secretary, Dr. S. V. EATON, Department of Botany, The University of Chicago, and make application for membership. Ten dollars will procure volume I while it lasts, and give the member all of the 1927 issues.

Fifth National Colloid Symposium.—The fifth National Colloid Symposium will be held at the University of Michigan, Ann Arbor, June 22–24, 1927. It has been customary to invite some distinguished investigator as the guest of honor. This year Dr. H. R. KRUYT, of the University of Utrecht, has been invited to lead the discussions. These meetings have been very stimulating in the past, and biologists will find much of value in the discussions. Anyone interested in colloidal phenomena is welcome to attend the meetings. There are no fees of any kind, and the fellowship is un-

usually cordial among the attendants of these symposia. Dr. KRUYT will remain at the University of Michigan for the summer session and will offer an opportunity for special courses in colloid chemistry.

Hydrogen Ion Concentration.—The second German edition of L. MICHAELIS'S *Wasserstoffionenkonzentration* has been translated into English by Dr. W. A. PERLZWEIG, of Johns Hopkins University, and thus a most excellent book becomes generally available to the students in this country. Already familiar to many of us in the German, the book needs no detailed description. The first part deals with the chemical equilibrium of the ions, and the second part with the ions, particularly the hydrogen ions, as sources of electric potential differences. Each part consists of five chapters. The book considers the theoretical problems of equilibrium and potential difference in a very helpful way, and supplements in an excellent manner the manual by CLARK. It is published by Williams and Wilkins, Baltimore, Md., in handsome binding. Price, \$5.00.

Manual of Plant Diseases.—The best text yet produced for the student of phytopathology has been written by Dr. F. D. HEALD, of the Washington State College. The book is also of considerable interest to plant physiologists because it contains a number of chapters on the so-called physiological diseases of plants. Section II is entitled "Non-parasitic Diseases," and contains chapters on diseases due to deficiencies of food materials in the soil; diseases due to excesses of soluble salts in the soil; diseases due to unfavorable water relations; diseases due to unfavorable air relations; diseases due to high temperatures; diseases due to low temperatures; diseases due to unfavorable light reactions; diseases due to manufacturing or industrial processes; and disease due to control practices. There is also a section on virus diseases. From this classification one can see that there is no such thing as an undiseased plant.

Physiologists will probably take the point of view that these matters belong, also, in texts on plant physiology. Certainly the material ought to be a part of the physiological work in every laboratory in the country. These diseases after all are only the "normal" response of the plant to the given environment, and progress in the elucidation of these physiological responses will be more rapid if well trained physiologists take an interest in the investigation and presentation of the facts regarding these causes of physiological breakdown. Physiologists have been too slow to take advantage of the practical and economic aspects of their work.

The book, which is excellently done, should be a possession of all who desire to keep abreast of the advances in the field of phytopathology. The price is \$7.00, and the publishers, McGraw-Hill Co.

Palladin's Plant Physiology.—The LIVINGSTON translation of this work has appeared in the third English edition. It is essentially the same as the second edition, but has had the editorial notes revised to include some more recent references. The list of cited literature at the beginning of the volume has been nearly doubled in size, and the book will no doubt continue to be useful to students of plant physiology. P. Blakiston's Son and Co. are the publishers, and the price is \$4.00.

Surface Chemistry.—Surface phenomena play a very large rôle in biological processes, and this "Introduction to Surface Chemistry," by Professor ERIC K. RIDEAL, Humphrey Owen Jones Lecturer in Physical Chemistry at Cambridge University, should be a very useful volume to the physiologist. The preface is by Dr. F. G. DONNAN, of University College, London, who emphasizes the importance of the "two dimensional" molecular world, the existence of which has been revealed by the pioneer work of Lord RAYLEIGH, MARCELIN, HARDY, LANGMUIR, ADAM, and RIDEAL. The survey of this rapidly developing field is admirably executed, and the book should be in the hands of all whose studies involve a knowledge of surface chemistry.

The first two chapters deal with surface tension of liquids, and solutions, while the third chapter discusses the surface films of insoluble materials. The succeeding three chapters take up the interface phenomena in liquid-liquid, gas-solid, and liquid-solid interfaces. The seventh chapter deals with differences of potential at interfaces, and chapter eight considers the problems of stability in suspensions and emulsions. The final chapter is devoted to gels and hydrated colloids, such as silica gel, gelatine, proteins, soaps, and colloidal dyes.

The book is published by the Cambridge University Press, and may be ordered from the Macmillan Co., New York. Price, \$5.50.

PLANT PHYSIOLOGY

APRIL, 1927

NITROGENOUS METABOLISM OF *PYRUS MALUS* L.

III. THE PARTITION OF NITROGEN IN THE LEAVES, ONE AND TWO YEAR BRANCH GROWTH AND NON-BEARING SPURS THROUGHOUT A YEAR'S CYCLE*

WALTER THOMAS
(WITH FOUR FIGURES)

Introduction

OSBORNE and his colleagues (23) in discussing the status of plant chemistry state: "It is true that we know a multitude of products derived from plants, and we know much of the chemistry of these, but this knowledge consists mostly of isolated facts which contribute comparatively little to a knowledge of the chemical make-up of the plant as a whole." This situation is due to the fact that the largest contributions to our knowledge of the compounds formed by plants have emanated from investigators in the chemical laboratories of large pharmaceutical manufacturing establishments who have vigorously prosecuted research in this direction for the purpose chiefly of isolating products of interest and value in therapeutics. Such investigations are not concerned with the energetics or dynamics of the plant, *i. e.*, with the functions and fate of the compounds isolated, in their relation to the response of the plant to the internal conditions existing during successive stages of their life cycle, by whatever means these may have been produced.

The theories of LIEBIG, though shown later to be false, spurred the early agricultural chemists and physiologists to carry out a prodigious amount

* Published with the approval of the Director of the Agricultural Experiment Station as scientific paper no. 428 and contribution no. 29 from the Department of Agricultural and Biological Chemistry.

of work on the quantitative changes of the various elements—ions—absorbed by the plant throughout the principal vegetative periods. Much of this work is abortive because the methods then used do not conform to modern standards of accuracy. The rapid advances during the last two decades in the chemistry of proteins and the products of their hydrolysis necessitates the investigation of the function of nitrogen with the application of this newer knowledge.

SCOPE AND LIMITATIONS OF THIS INVESTIGATION

The investigation of the dynamic systems produced by the large number of chemical reactions which have given rise to the various nitrogen compounds in the plant, and of the function of the individual groups in metabolism, is a laborious task involving much observational and analytical work. A quantitative measure of the changes undergone by the various nitrogen groups throughout a year's cycle is outlined in this paper. But what specific rôle is played by each in the metabolic cycle and in what manner their activity can be controlled are problems for further investigation. In the next paper it will be shown in what manner fertilizer application of an inorganic salt like NaNO_3 can modify the seasonal changes in some of the principal nitrogen fractions.

It is doubtful if, in the present state of our knowledge, all the data accumulated in these investigations can be interpreted. A great deal of information and insight, however, can be obtained with respect to the transformations taking place and also to the relationships of the various fractions to one another. The bearing of the results presented in this paper to practical horticulture has been presented in a previous report (37). It is of interest to note that no relationship could be discovered between either temperature or sunshine or rainfall or humidity and any of the nitrogen fractions.

The content of soluble nitrogenous constituents of the cells from aerial parts of *Pyrus malus* is relatively low (35); thus, only seven to ten per cent. (depending upon the season of the year) of the total nitrogen of the leaves is soluble in water. This is the case whether the soluble cell products are obtained by means of cytolytic agents or by direct extraction with water. In these investigations preference is given to the latter method (35). Since nothing was to be gained by any further investigation of the soluble cytoplasmic proteins (36), the next logical procedure appeared to be an examination of the metabolically active or reactive phases of the products of the nitrogen transformations. These are measured by quantitative determinations, during a year's cycle, of the fluctuations of the various groups of nitrogen fractions soluble in water. The relationships of these fractions to one another and to any external factor or factors have also been investigated.

CATALYSERS IN GROWTH AND REPRODUCTION

The classical investigations of EMMERLING (10) had indicated the relationship of amino-acids and amides to the synthesis of proteins, but their rôle in growth had not been stressed until MACDOUGAL and SPOEHR and MCGEE postulated that amino-acids and amides were the principal fractions influencing the rate of growth by functioning as catalytic agents, either by increasing the hydration capacity of the pentosan-protein colloids, as suggested by the former (21), or by accelerating the rate of carbohydrate katabolism, as indicated by the latter (33).

Although the investigation *in vitro* (even in a crude and rough fashion) of the course of definite reaction types, with the hope of discovering analogies between them and the complicated processes in the cell, may furnish valuable clues, nevertheless the extension and generalization of dogmatic interpretations and conclusions from experiments like MACDOUGAL'S, carried out *in vitro* to processes *in vivo*, must be made with considerable reservation. Moreover, in SPOEHR and MCGEE'S experiments dealing with the effect of feeding glycocoll on the stimulation of the rate of respiration and carbohydrate consumption, the plants and also excised leaves were subjected to more complete shading than would occur under normal growth conditions; hence, the conclusions drawn are hardly applicable to the normal processes of the plant. Again, the behavior of nitrogenous compounds as catalytic agents is not peculiar to amino-acids, for DAKIN (9) found that not only amino-acids but also peptone could be used to catalyse certain condensations at low temperatures, and KNOEVENAGEL (cited by DAKIN, *loc. cit.* p. 50) found that even organic bases (diethylamine, piperidine, aniline, and other bases) could be used to bring about a number of syntheses, at low temperatures, such as the union of aldehydes and peptones; and substances containing the $-\text{CO}-\text{CH}_2\cdot\text{CO}-$ group. Since the other nitrogen fractions, *viz.*, ammonia, amides, peptides, basic compounds and also those unclassified nitrogen compounds that do not respond to the tests in any scheme thus far developed for partitioning the complex nitrogenous compounds of plants—the so-called "rest" N fraction—are shown in these experiments to take part in the nitrogen transformation during the vegetative cycle, the specific rôle of each fraction in relation to the synthesis and degradation of proteins, and, therefore, in the growth and reproduction processes must also be considered. The present investigations indicate that these "rest" N compounds may play as important a rôle as the amino-acids. The action of catalyzers is attributed to their capacity to exist in different states of oxidation. Nitrogen, copper, manganese and iron, for this reason, are especially active catalytic agents. COOK (6) has given evidence from which one may deduce that iron and copper are the

prime oxidation catalysts in cell metabolism. The stressing of the importance of amino-acids has had the unfortunate effect of leading some to believe that determination of these acids may indicate or be a measure of the vigor of growth; but this hypothesis is not borne out in the present investigations. It is possible, therefore, that the "rest" N compounds and possibly other fractions may play a rôle equally as important as amino-acids.

Materials and methods

ELIMINATING VARIABLES.—Since the different parts of a fruit tree appear to behave as independent units (1, 19) and because variations of composition occur from the tip to the basal part of the shoot (39), the elimination of all controllable variables is essential. For this reason, in all experimental work involving analyses, fertilizers should be applied uniformly around the root system and care exercised to sample the tree uniformly. It is obvious, also, that if comparisons are to be made between two or more trees they should be of the same age and variety, growing in a homogenous soil. These principles were adhered to in these investigations.

DIFFICULTIES INHERENT TO ANALYTICAL INVESTIGATIONS IN PERENNIAL WOODY PLANTS.—HARVEY and MURNEEK (14) have discussed the analytical difficulties of experimental work with woody perennials such as fruit trees in comparison with herbaceous annuals like the tomato. Reference has been made to one of the disadvantages, *viz.*, the behavior of the different parts of fruit trees as independent units; another disadvantage lies in the relatively small percentage of active tissue present, in consequence of which special refinement in analytical methods is essential. Still another difficulty that is not encountered in herbaceous plants lies in the relative alteration in the proportions of wood to bark with age. The ratio of wood to bark varies from the apex to the base of the branch in the sense that it increases with the age of the growth. A determination of the *absolute* changes in magnitude of any group or constituent throughout the vegetative cycle is, therefore, precluded. However, relative fluctuations and especially the direction of these changes, as influenced by external factors, can be studied. The difficulty of the wood-to-bark ratio may, in part, be overcome by separating the bark from the wood and analyzing each separately; but even this method is not free from criticism, for during the interval required to separate the bark from the wood enzymatic changes can take place that might seriously affect the results. Analyses have been made both with and without separation in these investigations, with the result that no advantage is derived from such a separation. Moreover, the interpretation of results is not changed by the method of procedure in this respect, because, although the bark is higher than the wood in total nitro-

gen, it is lower in most of the water-soluble fractions; consequently, when the curves for amino-acids and amides, for example, are drawn for the wood and the bark separately throughout the cycle, the relative increase in mass of wood to bark is counterbalanced by the somewhat higher amounts of these fractions in the wood compared with the bark.

A Stayman Winesap tree, 15 years old, in the "off" bearing year, of known history and performance, growing in sod in the College Experimental Orchard, was used in this investigation. It received an application of 5 lbs. NaNO_3 on April 7, 1923. The desirability of restricting the work to one tree is obvious as a means of eliminating variability. Although a composite sample of a number of trees, provided they were of the same variety and age, if available, would give larger samples, thus facilitating analytical work, the possible advantages to be gained by this procedure would be counterbalanced by the disadvantages attendant upon the inability, in a preliminary investigation, to focus attention upon the behavior of one plant.

Samples of leaves, non-bearing spurs, and branches of three different ages (one year, two years, and three to seven years) were taken at eleven dates between June 18, 1923, and June 11, 1924. The sampling was done at times when the meteorological (rainfall and humidity) conditions had been favorable for three consecutive days preceding the taking of the samples. The diurnal variations which occur in the tissues of the apple tree were found to be small compared with the seasonal changes. They were avoided by always taking the samples at the same time of the day, in the early morning. Although a small decrease occurred in the total nitrogen at night, no changes could be observed in the total water-soluble products during this period.

FRACTIONATION METHODS

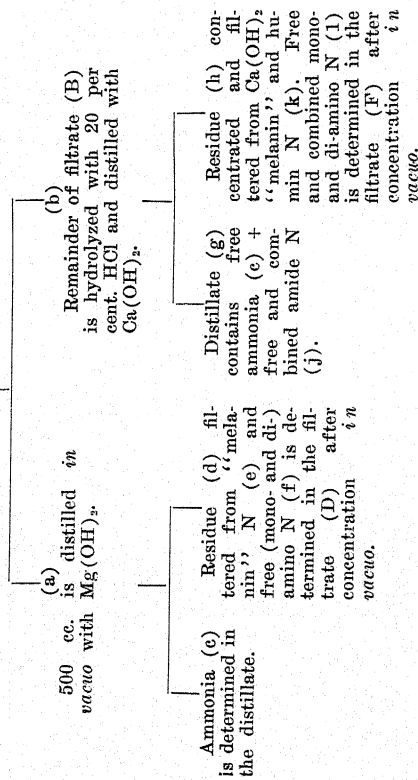
The method of extraction has already been described (35). Some investigators effect the extraction of nitrogenous constituents by means of boiling water. This method is, however, objectionable not only because a certain degree of peptonization of proteins may take place, but many nucleins are decomposed by boiling water with formation of hypoxanthine, etc. (30). Water at 40° C. was used for the extraction in this investigation.

Any method of separating the nitrogen fractions will necessarily be based upon the work of the army of investigators who have contributed to our knowledge of the analysis of proteins and their hydrolytic products. Among the contemporary workers who have sought to apply the nitrogen distribution methods to heterogeneous compounds of plant origin should be mentioned CHIBNALL, WILLIAMS, and the Illinois group

THE FRACTIONATION METHODS ADOPTED IN THESE INVESTIGATIONS

SCHEME I

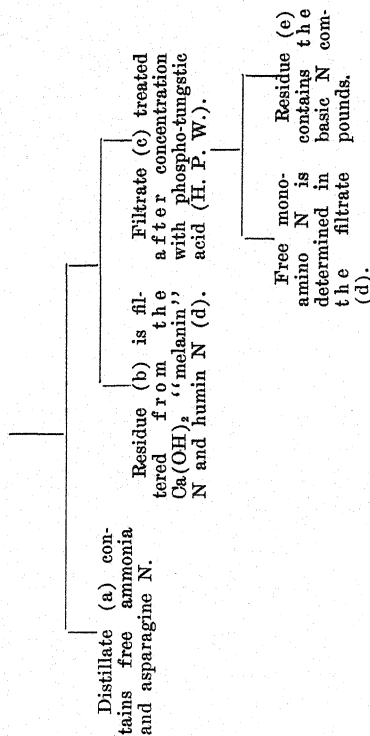
A 500 cc. aliquot of the non-protein N filtrate (B) (i.e., the filtrate from the colloidal ferric hydroxide precipitate) is treated according to (a). The remainder is hydrolyzed according to (b).



(l)-(f) gives combined mono- and di-amino N.

SCHEME II

The whole of the non-protein N filtrate (B) is taken and hydrolyzed with 4 per cent. HCl.



(HAMILTON, GRINDLEY, and colleagues), who have contributed numerous papers in this field.

Two different methods of separation have been used by the writer as a basis for the characterization of the various non-protein fractions. These two methods are, for clarity, represented here diagrammatically.

The two schemes differ with respect to the rigor of the hydrolysis, resulting in a differentiation with respect to (a) the distribution of the mono-amino- and the di-amino-acids, and (b) of the substances determined as amide N. Most of the complex peptide linkings would be decomposed by the stronger hydrolysis with 20 per cent. HCl, so that whereas the amide N in fractionation scheme I includes the combined as well as the free $-\text{CO}-\text{NH}_2$ groups, the mild hydrolysis adopted in fractionation scheme II gives as amide N only asparagine and other similarly constituted acid amides, *i. e.*, those having the $-\text{CO}-\text{NH}_2$ group free. Again in scheme I the free amino (both mono- and di-) and also the combined amino N (both mono- and di-) are determined *separately*, whereas in scheme II compounds containing the combined di-amino N groups are precipitated with the bases. The latter fractionation method will, therefore, give only the free mono-amino N.

A group of not very clearly defined nitrogenous compounds, *viz.*, the so-called "humins" and "melanins" fractions are separated in both fractionation methods. The origin of the "humins" N from the hydrolysis of proteins has been studied by GORTNER *et al* (11, 12, 13, 16). The term "melanin" N is used by the writer to denote the compounds precipitated by $\text{Ca}(\text{OH})_2$ or $\text{Mg}(\text{OH})_2$. It represents the nitrogen belonging to the acid pigment-like bodies found in the sap of plants. MORROW and GORTNER (22) believe that this nitrogen may also be derived from the calcium salts of the purine and pyrimidine bases. This, however, is questionable, for the barium salts of the pyrimidines are quite soluble as are also some of the alkali earth salts of the purines like thiobromine sodium.

As already mentioned, there remains in both fractionation schemes a group of nitrogen compounds, usually designated as "rest" N or "other" N, which do not respond to the tests in any scheme thus far developed for determining the partition products. This residual N cannot represent identical nitrogen compounds in the two methods. Thus, in scheme I the "rest" N will represent the difference between the total non-protein N and the sum of the ammonia, free (mono- and di-) amino, combined (mono- and di-) amino, free and combined amide and also "humins" nitrogen; while in scheme II, based on the HAUSMANN (15) separation of the mono- and di-amino acids by phosphotungstic acid (H. P. W.), the "rest" N will represent the difference between the total non-protein N and the sum of the ammonia, free mono-amino N, asparagine N (amide N) (28).

basic N and "humin" N. These basic nitrogen compounds comprise the derivatives of trimethyl-amine, hygrine, adenine, choline, betaine, stachydrine; the purine bases; and also the basic di-amino compounds like arginine, histidine, lysine and cystine.

The first fractionation scheme then determines both mono- and di-amino acids, but the bases and di-amino acids are removed by phosphotungstic acid in the second fractionation method, and which, accordingly, estimates only the mono-amino acids. It will be shown in a later paper that the "rest" N compounds of scheme II are higher throughout the cycle than those of scheme I. This indicates (a) that the peptide combinations present in these filtrates are not precipitated by phosphotungstic acid, though doubtless, as found by VICKERY (43), some polypeptides may be precipitated by phosphotungstic acid; or (b) that the combined mono- and di-amino N compounds (*i. e.*, compounds containing peptide linkages) are present in the non-protein fraction in much larger amounts than the basic nitrogen; or (c) that a portion of the "rest" N fraction has undergone decomposition during the more vigorous hydrolyses adopted in scheme I. Thus, CHIBNALL (3) found in his starvation experiments that hydrolysis with strong acids completely decomposed the "rest" N compounds, half their nitrogen appearing as ammonia N and about one-third as bases.

Without going into details respecting the nature of the substances precipitated by phosphotungstic acid, from the literature of the subject (18, 24, 31), it is certain that a variable product is obtained, depending to some extent on the concentration of the solution, and *even in the case of the decomposition products of pure proteins*, other decomposition products besides the purines and the di-amino acids, histidine, arginine, and lysine appear to be precipitated by phosphotungstic acid, according to conditions. Probably leucine and tyrosine may also be precipitated (31). KIESEL (18) under the conditions of precipitation used by him found both phenylalanine and aspartic acid in the phosphotungstic acid precipitate. These results are undoubtedly due to the working conditions. Since the phosphotungstates of some amino acids are relatively insoluble, as a precautionary measure, it is always desirable to dilute to a large volume before attempting separation of the bases (42).

The first fractionation scheme was adopted in the 1923-24 experiments which are described in this paper and the second scheme in the experiments to be described in a later paper. Although undoubtedly superior to the second scheme, from the standpoint of accuracy, the first method possesses the disadvantage of being time-consuming, owing to the additional separations required.

DETAILS OF ANALYTICAL PROCEDURE

Attention is called at the outset to the fact that nitric N was found only at one period—just as the buds are opening. This point has been described fully elsewhere (38).

(a) AMMONIA N.—This includes the ammonia of ammonium salts and is usually described in the literature as “free” ammonia for the purpose of differentiating between this ammonia and that formed by the hydrolysis of amides. Inasmuch as the protein-iron gel (35) is more readily filtered if heated, and inasmuch as a slight decomposition of peptides occurs under certain not well-defined conditions, it is advisable to determine ammonia on a 200 cc. aliquot of the *original extract* denoted as (A), before removal of the water-soluble proteins. The determination is best carried out in the apparatus and by the method described by VAN SLYKE (40). It is only fair to state here, since it is not generally known, that the method for ammonia N is essentially that described by LONGI (20), who distilled at a temperature of 38-40° C. with $Mg(OH)_2$. He noted that neither urea nor asparagine were decomposed under these conditions. The writer prefers solid CaO (26) to MgO for the reason described later.

(b) FREE MONO- AND DI-AMINO N.—The removal of the “free” ammonia must be first carried out as described under (a) *from a 500 cc. aliquot of the filtrate* previously freed from soluble proteins in the manner already described (35). This filtrate freed from proteins is denoted as (B.) CaO is now better substituted for MgO. It is advantageous to use only a very slight excess of *solid* CaO, the exact amount being determined by a preliminary titration (26). With this precaution little or no frothing occurs in the VAN SLYKE apparatus during the determination of amino N. Pure CaO is recommended in preference to MgO because in the experience of the writer the former occludes or absorbs more of the pigments to which most of the frothing can be attributed. No success was obtained with secondary octyl (caprylic) alcohol (even twice redistilled, B. P. 131° C.) which has been recommended for the purpose of reducing the surface tension and, consequently, the frothing (41). The blanks from this reagent, due presumably to the evolution of some gas that is not absorbed by the alkaline permanganate mixture, were found too high to justify its use in the determination of the relatively small amount of amino N measured in these experiments.

A small and somewhat variable quantity of *nitrogenous matter* will always be *precipitated*, occluded or absorbed by the CaO. This is “melanin” N and is removed by filtration and washed with hot water; the filtrate, which is denoted as (C), is acidified with acetic acid and afterwards con-

centrated under a pressure of 15 mm. to about 15 cc. using a CLAISSEN flask (40). In this concentrated solution a further precipitate containing Si, Al, Ca, Na and K salts usually separates out. This precipitate is removed by filtration and the filtrate, which is denoted as (D), made up to a definite volume prior to its introduction into the VAN SLYKE micro-apparatus (40, 41).

The writer has confirmed SPOEHR and MCGEE's observations (32) with respect to the alleged interference of carbohydrates in the nitrous acid method (cited by SPOEHR and MCGEE, *loc. cit.* p. 34, footnote). The determination of the amino N in aspartic acid was not altered by the presence of glucose or fructose.

(c) FREE AND COMBINED AMIDE N.—A definite volume of concentrated HCl sufficient to give a 20 per cent. solution is now added to the remainder of the original filtrate (B) containing the non-protein nitrogenous constituents, *i.e.*, to the extract remaining after the removal of the aliquots for the determination of free and combined amino N as under (b) above. The mixture is then hydrolyzed for twelve hours with the usual precautions; after the removal of the HCl *in vacuo* (40) the residue is taken up in water and distilled *in vacuo* with a slight excess of solid CaO. A titration of the distillate gives both the "free" ammonia N and the "free and combined" amide N. The difference between the titration values for ammonia obtained as described under (a) and (c) gives the "free and combined" amide N. The solution still remaining in the flask is denoted as (E).

(d) COMBINED MONO- AND DI-AMINO N.—The solution (E) left in the flask from the determination of the free and combined amide N (c) is filtered from the CaO "melanin" nitrogenous material, washed, and the combined filtrates and washings (F) acidified with acetic acid and concentrated *in vacuo* to a volume of about 15 cc. A determination of the amino N in this gives the "free" and also the "combined" amino N. The latter, which is indicative of the complexity of the peptide linkings, is determined by the difference in the values obtained in this determination and that given under (b).

Experimental results

The results of the analyses by the foregoing methods are given in the following tables. Table I gives the percentages of imbibitional and total water in branches of different ages, and in the short spurs and leaves. Table II shows the water-soluble nitrogenous constituents of the leaves, branches of different ages, and short spurs. The partition of the non-protein fraction is shown for various collecting dates in percentages of the green weight and dry weight.

TABLE I
THE PERCENTAGES OF IMBIBITIONAL AND TOTAL WATER
ONE-YEAR BRANCH GROWTH

SERIES NO.	COLLECTING DATE	FRESH WEIGHT	DRY WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
		gm.	gm.	Per cent.	Per cent.
1	June 18, 1923	55.0	26.3	52.1	57.6
7a	July 16, 1923	102.0	50.7	50.3	55.3
15	August 17, 1923	41.8	20.5	51.0	56.5
27	September 17, 1923	129.0	60.0	53.5	56.7
34	October 15, 1923	104.0	52.5	50.0	54.7
46	November 20, 1923	125.0	66.2	47.0	51.8
56	April 12, 1924	120.0	62.0	48.3	54.0
64	April 21, 1924	80.0	40.0	50.0	54.0
80	April 28, 1924	69.0	34.3	50.7	54.0
98	May 13, 1924	90.0	40.0	55.6	57.9
118	May 22, 1924	57.0	23.0	59.6	61.0
143	June 11, 1924	56.0	23.8	57.5	58.0

TWO-YEAR BRANCH GROWTH

SERIES NO.	COLLECTING DATE	FRESH WEIGHT	DRY WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
		gm.	gm.	Per cent.	Per cent.
03	June 18, 1923	75.0	35.4	52.8	54.2
7	July 16, 1923	150.0	75.0	50.0	54.0
19	August 17, 1923	69.5	33.8	52.0	54.5
31	September 17, 1923	167.0	87.0	47.9	53.7
38	October 15, 1923	155.0	85.0	45.2	50.7
47	November 20, 1923	161.0	87.0	44.7	49.8
57	April 12, 1924	65.0	35.1	46.0	48.0
65	April 21, 1924	82.0	45.0	45.1	51.1
97	April 28, 1924	125.0	67.0	46.4	52.4
119	May 22, 1924	132.0	65.0	50.8	54.8
147	June 11, 1924	140.0	70.0	50.0	53.8

OLDER GROWTH

SERIES NO.	COLLECTING DATE	FRESH WEIGHT	DRY WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
		gm.	gm.	Per cent.	Per cent.
4	June 18, 1923	210.0	110.4	47.4	52.3
8a	July 16, 1923	230.0	118.4	48.4	52.3
14a	August 17, 1923	199.1	101.0	49.3	51.6
32	September 17, 1923	174.0	98.0	48.0	50.2
39	October 15, 1923	632.0	297.0	47.0	50.1
49	November 20, 1923	455.0	244.0	47.0	50.2
58	April 12, 1924	367.0	202.2	44.9	50.6
66	April 21, 1924	165.0	89.0	46.1	49.5
82	April 28, 1924	239.0	133.0	44.3	51.7
99	May 13, 1924	196.0	100.0	48.9	53.2
121	May 22, 1924	183.0	96.0	47.5	53.1
148 } 148a }	June 11, 1924	{ 419.0 { 127.0	{ 236.0 { 58.0	{ 43.7 { 54.3	52.4

SHORT SPURS

SERIES NO.	COLLECTING DATE	FRESH WEIGHT	DRY WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
		gm.	gm.	Per cent.	Per cent.
5	June 18, 1923	31.0	15.1	51.3	57.0
7	July 16, 1923	35.0	16.8	52.0	55.3
17	August 17, 1923	26.5	11.4	47.0	53.0
29	September 17, 1923	37.0	20.2	47.5	51.2
37	October 15, 1923	121.0	65.2	46.3	51.3
48	November 20, 1923	72.0	31.5	44.0	48.8
63	April 12, 1924	58.0	30.0	48.3	54.0
69	April 21, 1924	54.0	26.5	51.9	57.5
83	April 28, 1924	38.0	19.0	50.0	58.7
96	May 13, 1924	15.0	8.0	46.7	50.8
122	May 22, 1924	22.0	9.0	49.1	lost
149 } 150 }	June 11, 1924	{ 2.5 { 5.5	{ 1.9 { 2.7	{ 24.0 { 50.9	46.0

LEAVES

SERIES NO.	COLLECTING DATE	FRESH WEIGHT	DRY WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
		gm.	gm.	Per cent.	Per cent.
01 } 001 }	June 18, 1923	38.0	17.2	54.8	57.0
6 } 6a }	July 16, 1923	242.0	111.2	54.1	63.0
18 } 20 }	August 17, 1923	127.0	51.8	59.8	65.5
28 } 30 }	September 17, 1923	406.0	185.1	52.4	59.0
35 } 36 }	October 15, 1923	516.0	245.0	52.9	60.5
50	November 20, 1923	95.0	48.4	49.1	55.2
100	May 13, 1924	47.0	13.0	72.3	75.0
119a } 123 }	May 22, 1924	72.0	lost	lost	lost
144	June 11, 1924	50.2	18.6	62.5	66.5
205	August 17, 1924	150.0	58.2	61.2	65.0

ACCURACY AND LIMITS OF ERROR

The errors of sampling could not be determined on account of the necessity for conserving the material, but the determination of the accuracy and limits of error of the analytical data has been made by expressing, as a percentage of the mean, the differences obtained in certain duplicate analyses. These are given in table III.

The total N shows a maximum difference of 0.58 per cent., the water-soluble N of 1.50 per cent., and the "free" mono- and di-amino N of 5.20 per cent. The experimental error in the "free" amino N is higher than that of the total nitrogen and total water-soluble N, because of the smaller amount of nitrogen determined.

Since the amide N is determined from the difference between the total NH_3 before and after hydrolysis, this determination will carry the errors of the ammonia determination also. It shows a maximum difference of 7.15 per cent. From the foregoing it is clear that all fluctuations in the nitrogen fractions can be regarded as significant.

Discussion and conclusions

(a) TOTAL NITROGEN

In general, the total nitrogen of the leaves (fig. 1) follows the course found for this species by other investigators (2, 17); these changes will, therefore, not be elaborated upon here, except to point out that the change

TABLE II

AMOUNT OF WATER-SOLUBLE NITROGENOUS SUBSTANCES PRESENT IN THE LEAVES, 1923 BRANCH GROWTH, 1922 BRANCH GROWTH, SHORT SPURS, AND THE PARTITION OF THE NON-PROTEIN PORTION EXPRESSED AS PERCENTAGES OF FRESH AND DRY WEIGHTS, RESPECTIVELY

LEAVES

SERIES NO.	COLLECTING DATE	TOTAL N	PROTEIN N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	NH ₃ N	HUMIN N	FREE AMINO N	COMBINED AMINO N	AMIDE N	RESIDUAL N
				As percentages of fresh weight of material							
4A	June 18, 1923	0.719	0.0047	0.0606	0.0559	0.0013	0.0112	0.0162	0.0127	0.0094	0.0051
6	July 16, 1923	0.691	0.0030	0.0510	0.0482	0.0017	0.0087	0.0104	0.0126	0.0081	0.0066
20	August 17, 1923	0.641	0.0027	0.0430	0.0403	0.0024	0.0091	0.0070	0.0119	0.0024	0.0075
28	September 17, 1923	0.715	0.0038	0.0570	0.0532	0.0059	0.0119	0.0156	0.0176	0.0016	0.0006
35	October 15, 1923	0.646	0.0019	0.0422	0.0405	None	0.0022	0.0120	0.0135	0.0086	0.0040
50	November 20, 1923	0.585	0.0009	0.0280	0.0271	None	0.0013	0.0034	0.0027	0.0106	0.0091
100	May 13, 1924	0.877	0.0110	0.0820	0.0710
144	June 11, 1924	0.671	0.0060	0.0410	0.0350
				As percentages of dry weight of material							
4A	June 18, 1923	1.656	0.0110	0.1410	0.1300	0.0032	0.0261	0.0376	0.0300	0.0219	0.0120
6	July 16, 1923	1.869	0.0100	0.1370	0.1270	0.0046	0.0270	0.0281	0.0345	0.0177	0.0180
20	August 17, 1923	1.860	0.0076	0.1227	0.1151	0.0070	0.0250	0.0200	0.0341	0.0070	0.0220
28	September 17, 1923	1.746	0.0095	0.1425	0.1350	0.0147	0.0298	0.0390	0.0440	0.0044	0.0010
35	October 15, 1923	1.617	0.0048	0.1055	0.1013	None	0.0055	0.0300	0.0330	0.0215	0.0103
50	November 20, 1923	1.570	0.0020	0.0622	0.0600	None	0.0028	0.0075	0.0060	0.0239	0.0200
100	May 13, 1924	3.512	0.0440	0.3280	0.2840
144	June 11, 1924	2.000	0.0530	0.1206	0.0676

TABLE II—(Continued)

AMOUNT OF WATER-SOLUBLE NITROGENOUS SUBSTANCES PRESENT IN THE LEAVES, 1922 BRANCH GROWTH, 1922 BRANCH GROWTH, SHORT SPURS, AND THE PARTITION OF THE NON-PROTEIN PORTION EXPRESSED AS PERCENTAGES OF FRESH AND DRY WEIGHTS, RESPECTIVELY

1923 BRANCH GROWTH

SERIES NO.	COLLECTING DATE	TOTAL N	PROTEIN N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	NH ₃ N	HUMIN N	FREE AMINO N	COMBINED AMINO N	AMIDE N	RESIDUAL N
				As percentages of fresh weight of material							
1	June 18, 1923	0.269	0.0086	0.0507	0.0421	0.0012	0.0130	0.0125	None	0.0077	0.0077
5	July 16, 1923	0.324	0.0248	0.0739	0.0014	0.0014	0.0246	0.0175	None	0.0033	0.0022
15	August 17, 1923	0.288	0.0119	0.0717	0.0602	0.0092	0.0098	0.0185	0.0016	0.0057	0.0114
27	September 17, 1923	0.233	0.0025	0.0246	0.0221	0.0007	0.0051	0.0050	Trace	0.0052	0.0060
34	October 15, 1923	0.297	0.0066	0.0337	0.0271	0.0011	0.0039	0.0055	0.0057	0.0101	0.0008
46	November 20, 1923	0.328	0.0079	0.0451	0.0372	0.0020	0.0079	0.0079	None	0.0060	0.0134
56	April 12, 1924	0.272	0.0091	0.0268	0.0177	None	0.0023	0.0039	None	0.0088	0.0025
64	April 21, 1924	0.305	0.0142	0.0414	0.0262	None	0.0031	0.0105	None	0.0048	0.0012
80	April 28, 1924	0.304	0.0121	0.0576	0.0483	0.0031	0.0184	0.0114	0.0061	0.0054	0.0037
98	May 13, 1924	0.244	0.0071	0.0385	0.0280	0.0016	0.0026	0.0088	0.0075	0.0075
118	May 22, 1924	0.238	0.0027	0.0230	0.0203	0.0013	0.0023	0.0072	0.0013	0.0068	0.0013
144 } 144A }	June 11, 1924	0.249	0.0056	0.0302	0.0245	0.0009	0.0047	0.0099	0.0024	0.0052	0.0015
				As percentages of dry weight of material							
1	June 18, 1923	0.372	0.0200	0.1180	0.0980	0.0028	0.0302	0.0290	None	0.0180	0.0180
5	July 16, 1923	0.326	0.0552	0.1642	0.1090	0.0031	0.0546	0.0390	0.0073	0.0050
15	August 17, 1923	0.352	0.0261	0.1360	0.1369	0.0209	0.0223	0.0420	0.0037	0.0210	0.0260
27	September 17, 1923	0.367	0.0060	0.0600	0.0540	0.0018	0.0124	0.0121	Trace	0.0130	0.0146
34	October 15, 1923	0.381	0.0146	0.0750	0.0604	0.0024	0.0086	0.0123	0.0126	0.0225	0.0017
46	November 20, 1923	0.467	0.0165	0.0939	0.0774	0.0041	0.0164	0.0165	None	0.0124	0.0280
56	April 12, 1924	0.419	0.0198	0.0582	0.0384	None	0.0050	0.0088	None	0.0191	0.0054
64	April 21, 1924	0.380	0.0332	0.0900	0.0568	None	0.0177	0.0206	None	0.0105	0.0080
80	April 28, 1924	0.347	0.0262	0.1252	0.1050	0.0068	0.0400	0.0248	0.0132	0.0117	0.0084
98	May 13, 1924	0.315	0.0239	0.0874	0.0637	0.0037	0.0060	0.0200	0.0170	0.0170
118	May 22, 1924	0.340	0.0060	0.0512	0.0452	0.0028	0.0050	0.0160	0.0030	0.0150	0.0029
144 } 144A }	June 11, 1924	0.398	0.0120	0.0642	0.0522	0.0020	0.0100	0.0210	0.0050	0.0110	0.0032

TABLE II—(Continued)

AMOUNT OF WATER-SOLUBLE NITROGENOUS SUBSTANCES PRESENT IN THE LEAVES, 1922 BRANCH GROWTH, 1922 BRANCH GROWTH, SHORT SPURS, AND THE PARTITION OF THE NON-PROTEIN PORTION EXPRESSED AS PERCENTAGES OF FRESH AND DRY WEIGHTS, RESPECTIVELY

1922 BRANCH GROWTH

SERIES NO.	COLLECTING DATE	TOTAL N	PROTEIN N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	NH ₃ N	HUMIN N	FREE AMINO N	COMBINED AMINO N	AMIDE N	RESIDUAL N
				As percentages of fresh weight of material							
03	June 18, 1923.....	0.230	0.0110	0.0321	0.0211	0.0009	0.0055	0.0059	None	0.0055	0.0033
8	July 16, 1923.....	0.293	0.0179	0.0621	0.0442	0.0014	0.0224	0.0087	0.0067	0.0030	0.0020
19	August 17, 1923.....	0.221	0.0139	0.0389	0.0249	0.0040	0.0019	0.0049	0.0045	0.0046	0.0050
31	September 17, 1923.....	0.211	0.0047	0.0162	0.0113	None	0.0022	0.0025	Trace	0.0051	0.0014
38	October 15, 1923.....	0.240	0.0089	0.0261	0.0172	0.0012	0.0027	0.0054	0.0012	0.0089	0.0035
47	November 20, 1923.....	0.273	0.0131	0.0466	0.0335	None	0.0035	0.0080	0.0034	0.0060	0.0075
				As percentages of dry weight of material							
03	June 18, 1923.....	0.719	0.0240	0.0700	0.0460	0.0020	0.0120	0.0130	None	0.0120	0.0070
8	July 16, 1923.....	0.691	0.0390	0.1350	0.0960	0.0030	0.0488	0.0190	0.0146	0.0066	0.0040
19	August 17, 1923.....	0.641	0.0300	0.0836	0.0536	0.0086	0.0042	0.0106	0.0096	0.0100	0.0106
31	September 17, 1923.....	0.715	0.0100	0.0350	0.0253	None	0.0048	0.0065	Trace	0.0110	0.0030
38	October 15, 1923.....	0.646	0.0180	0.0530	0.0350	0.0024	0.0055	0.0110	0.0025	0.0180	0.0070
47	November 20, 1923.....	0.585	0.0260	0.0928	0.0668	None	0.0070	0.0160	0.0068	0.0120	0.0150

TABLE II—(Concluded)

AMOUNT OF WATER-SOLUBLE NITROGENOUS SUBSTANCES PRESENT IN THE LEAVES, 1923 BRANCH GROWTH, 1922 BRANCH GROWTH, SHORT SPURS, AND THE PARTITION OF THE NON-PROTEIN PORTION EXPRESSED AS PERCENTAGES OF FRESH AND DRY WEIGHTS, RESPECTIVELY

SHORT SPURS

SERIES NO.	COLLECTING DATE	TOTAL N	PROTEIN N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	NH ₃ N	HUMIN N	FREE AMINO N	COMBINED AMINO N	AMIDE N	RESIDUAL N
				As percentages of fresh weight of material							
2	June 18, 1923.....	0.372	0.0140	0.0355	0.0215	0.0013	0.0032	0.0047	None	0.0086	0.0037
7	July 16, 1923.....	0.326	0.0226	0.0502	0.0274	0.0019	0.0033	0.0104	None	0.0108	0.0009
17	August 17, 1923.....	0.352	0.0111	0.0959	0.0848	0.0230	0.0030	0.0199	0.0290	0.0029	0.0066
29	September 17, 1923.....	0.367	0.0098	0.0335	0.0237	0.0029	0.0036	0.0079	None	None	0.0093
36	October 15, 1923.....	0.381	0.0102	0.0510	0.0407	0.0041	0.0104	0.0147	None	0.0080	0.0034
48	November 20, 1923.....	0.467	0.0051	0.0446	0.0394	None	0.0102	0.0107	0.0037	0.0012	0.0134
				As percentages of dry weight of material							
2	June 18, 1923.....	0.844	0.0325	0.0825	0.0500	0.0030	0.0073	0.0110	None	0.0200	0.0087
7	July 16, 1923.....	0.726	0.0505	0.1115	0.0610	0.0042	0.0077	0.0231	None	0.0240	0.0020
17	August 17, 1923.....	0.752	0.0236	0.2040	0.1804	0.0489	0.0063	0.0423	0.0618	0.0061	0.0140
29	September 17, 1923.....	0.749	0.0200	0.0684	0.0484	0.0080	0.0073	0.0161	None	None	0.0190
36	October 15, 1923.....	0.780	0.0209	0.1040	0.0831	0.0084	0.0213	0.0300	None	0.0164	0.0070
48	November 20, 1923.....	0.916	0.0099	0.0857	0.0758	None	0.0197	0.0207	0.0072	0.0024	0.0258

TABLE III

DIFFERENCES OBTAINED IN CERTAIN DUPLICATE DETERMINATIONS AS PERCENTAGE
OF THE MEAN

SERIES NO.	TOTAL N	TOTAL WATER-SOLUBLE N	MONO- AND DI-AMINO N	AMIDE N
6	0.58	1.20	5.20	6.00
15	0.44	1.40	3.40	3.10
19	0.50	0.35	1.80	2.00
28	0.32	0.00	4.00	7.15
34	0.00	0.50	0.25	2.30
48	0.18	1.50	3.40	0.80
98	0.32	0.75	3.80	1.50

in direction observed in September has occurred at approximately the same period every year the experiments were in progress. It is probably due to the diminished requirement of the branch growths for the nitrogen synthesized by the leaves.

THE EVACUATION OF NITROGEN FROM THE LEAVES IN THE FALL.—This so-called "Rückwanderung" or autumnal evacuation of nitrogen from the leaves to the branches in the fall has been asserted and as vigorously denied (8). Much of the difficulty in interpretation, however, can be attributed to the fact that in many instances attempts have been made to draw conclusions from analysis of the leaves only, omitting analysis of the perennial parts. Both COMBES (4, 5) and RIPPEL (27) have recently reported an extensive study of this phenomenon in young oak and beech trees. According to the former, the migration of nitrogenous substances commences as soon as the leaves turn yellow, slowly at first and then more rapidly. This is not in agreement with the results of RIPPEL (*loc. cit.*) nor of the writer; these latter clearly indicate that the evacuation of nitrogen in the leaves is not initiated suddenly at the onset of yellowing but much earlier. If the decrease commenced just as soon as yellowing occurred it might be argued that, since the nitrogen present in the leaves depends upon the difference between the amount of protein synthesized and that utilized in growth and respiration, and that, inasmuch as protein synthesis decreases in the autumn owing to the degeneration of the chloroplasts, this affords an explanation of the migration provided removal goes on at the normal rate. However, since there is sufficient evidence (*loc. cit.*) to indicate that the decrease in nitrogen may occur before degeneration of the chloroplasts occurs, this migration cannot be due to the same causes as that which occurs normally during the life of the tree, but must be distinct from the normal process. Admittedly, it is

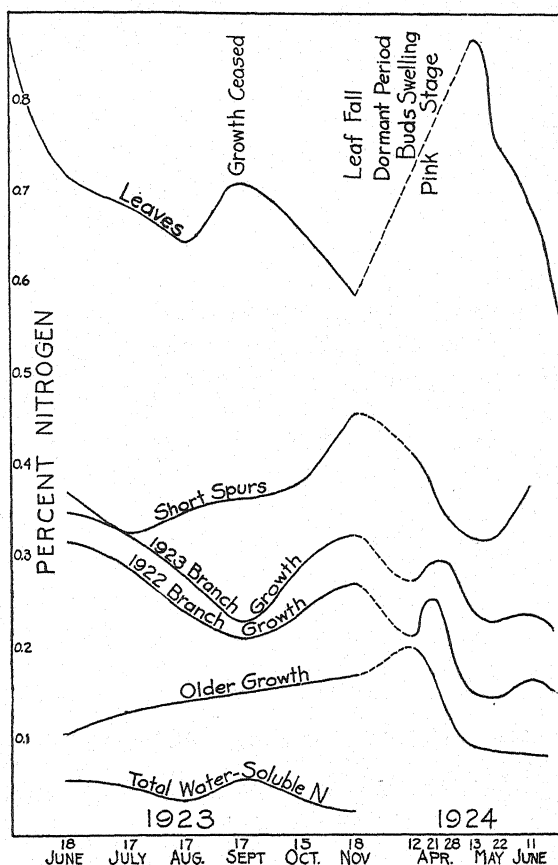


FIG. 1. Total nitrogen of the leaves, short non-bearing spurs, one-year, two-year and older branch growth as percentages of the fresh weight. June, 1923, to June, 1924.

difficult to understand. SCHERTZ (29) has shown that a draft on the nitrogen of the leaf by the younger tissues leads to a decomposition of nitrogen compounds of the leaf including proteins, phospho-lipines and chlorophyll, the decomposition of the latter producing mottling. With the loss of chlorophyll accompanying autumnal coloration, the yellow pigments increase at its expense due to the combination of the phytol that is split off from the chlorophyll with sugars to form carotin and xanthophyll (cited by SCHERTZ, *loc. cit.* p. 124). There can be no doubt, however, that whatever the cause, the present investigations confirm the existence of this migration phenomenon. The concurrent increase in nitrogen during this period in the short spurs, one year, two year and older branch growth,

which results from the evacuation from the leaves can readily be seen from an inspection of the analyses and fig. 1. The actual increase is probably greater than that shown if one allows for the relative increase of wood to bark. When the phenomenon of autumnal migration of nitrogen is considered in relation to the great demand of the shoots in the spring, as discussed above, this whole process may be looked upon as an expression of nature's method of conservation in the use of nitrogen.

THE PERIOD OF VEGETATIVE GROWTH.—Many investigators (9) have observed that a large decrease of nitrogen occurs in the older parts during the initiation of vegetative growth. Fig. 1 shows that during the period between the beginning of vegetative growth and the blossoming of the tree the loss of nitrogen from the branches is very large. The development of the buds and the formation of the new leaves are dependent upon the "reserve" proteins accumulated in the branches; a great expenditure of nitrogen compounds is thus required for growth of these parts. In some of the older tissues over half of the available stored nitrogen supply is given up to the young shoots, leaving a deficiency of nitrogen in these organs until this is supplied by the activity of the roots later in the spring.

A consideration of composite curves discussed elsewhere (37), showing the sum of the percentages of nitrogen in the leaves and in the one to seven year old branch growths, indicates that after *active* growth has ceased (third week in June) a condition of nitrogen equilibrium exists in the perennial parts until fall migration of nitrogen from the leaves commences.

CHANGES DURING THE DORMANT PERIOD.—The so-called dormant period is obviously accompanied by certain metabolic changes, for during this period with a mean daily temperature of 33.5° F. (the mean maximum being 35.5° F. and the mean minimum 20.2° F.) the total nitrogen in the short non-bearing spurs, one-year and two-year wood, decreased 11, 15 and 20 per cent., respectively.

During the commencement of the period of sap flow the nitrogen increased nearly 10 per cent. in the one-year wood and 18 per cent. in the two-year wood, and then during the rapid development of the shoot the nitrogen decreases rapidly in all parts due to the withdrawal of nitrogen stored in the phloem, as already stated. Compared with the quantities found when the tree entered the rest period, this amounts to a decrease of 32 per cent., 29 per cent., 21 per cent. and 22 per cent., respectively, in the short spurs, one-year branch growth, two-year branch growth, and older growth.

The form in which this decrease takes place cannot be measured by the changes in the water-soluble fractions taken at two periods only, because these are too small to account for the decrease involved. Apparently, there must be a continual degradation of the insoluble cytoplasmic proteins and

a transference to the older perennial parts throughout the whole dormant period.

(b) PARTITION OF NITROGEN IN THE LEAVES

In the leaves, the fluctuations (see figures 2 and 3) of the water-soluble, the non-protein, and also of the free and combined amino-nitrogen fractions vary inversely to their course in the branch growth throughout the whole cycle. The amide N does not appear to follow this or any other definite relationship. This inverse relationship in the leaves and branches between the groups mentioned is difficult to interpret because, although it is possible to express the data from the leaves on both an absolute and a percentage basis, the results on the branch growth cannot be so stated unless the entire tree is taken for analysis and this is impracticable in experiments of this type. Fig. 3 shows that the total water-soluble, the free (mono- and di-amino), and also the free and combined amide nitrogen parallel the total nitrogen of the leaves throughout the cycle; the "rest" N usually varies inversely to them.

Although the amide N (free and combined) and also the "rest" N decrease in the fall during the period of migration of nitrogen from the leaves to the branches, the other fractions, *e.g.*, the total water-soluble, the

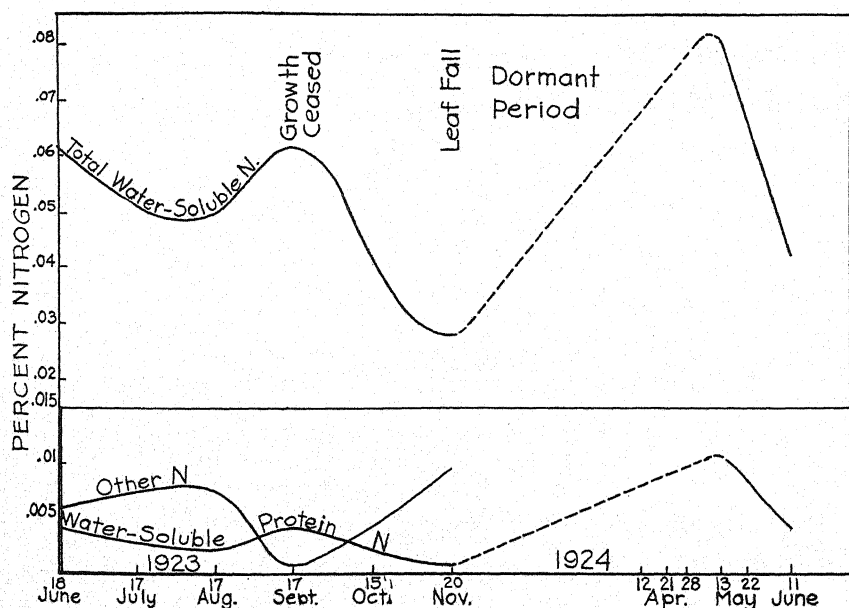


FIG. 2. Total water-soluble N, water-soluble protein N, and "rest" N of the leaves as percentages of the fresh weight.

non-protein, and the free (mono- and di-) amino-nitrogen decrease rapidly. The significance of this, to which reference will be made again, indicates that the "rest" N may have a close relation to protein degradation in the leaves (3). It is possible that nitrogen is translocated in the form of these "rest" N compounds. The water-soluble nitrogen is low throughout the cycle and varies inversely to the "rest" N. The significance of this is apparent.

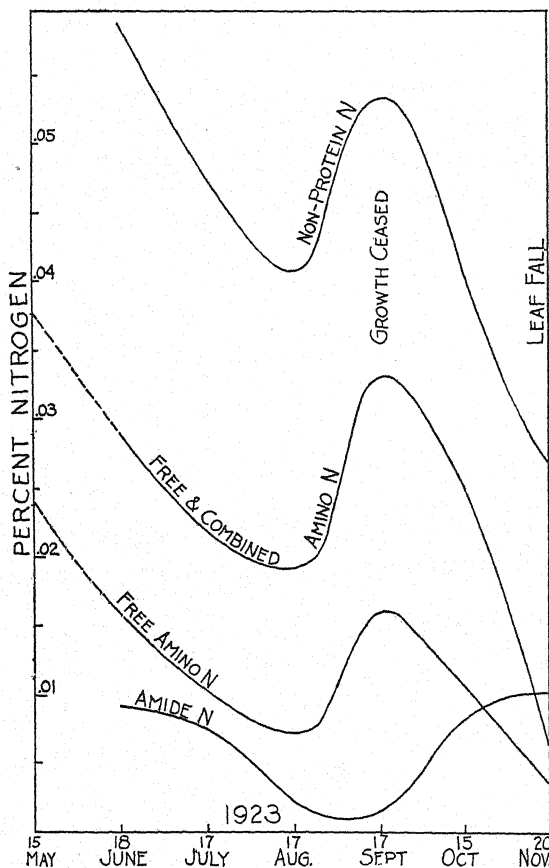


FIG. 3. The non-protein N, free and combined amino N, free amino N and amide N of the leaves as percentages of the fresh weight.

(c) PARTITION OF NITROGEN IN THE BRANCH GROWTH

The decrease in total nitrogen in the one- and two-year branch growth during the period of mobilization of reserve proteins (bud swelling) is accompanied by a rapid rise in the total water-soluble non-protein nitrogen, in the free amino, and also in the "rest" nitrogen fractions, the amide N

decreasing during this period (fig. 4). Possibly the increase in the amino N at this time may indicate that translocation in the flowing sap takes place during this period as amino-acids. That the transportation of the reserve material to the tissues during the germination of wheat seedlings takes place in large measure as free amino-acids has been shown by PETTIBONE and KENNEDY (25) and is thus analogous to the manner in which the proteins of the blood stream are transported to the tissues of animals. However, the fact that the "rest" N also parallels the amino N in magnitude and direction at this period indicates the importance of the rôle that these unknown fractions may also play. Both CHIBNALL and PRIANISCHNIKOW (see ref. 43, p. 660) affirm that asparagine is the form in which nitrogen is translocated from one part of the plant to another, but, as

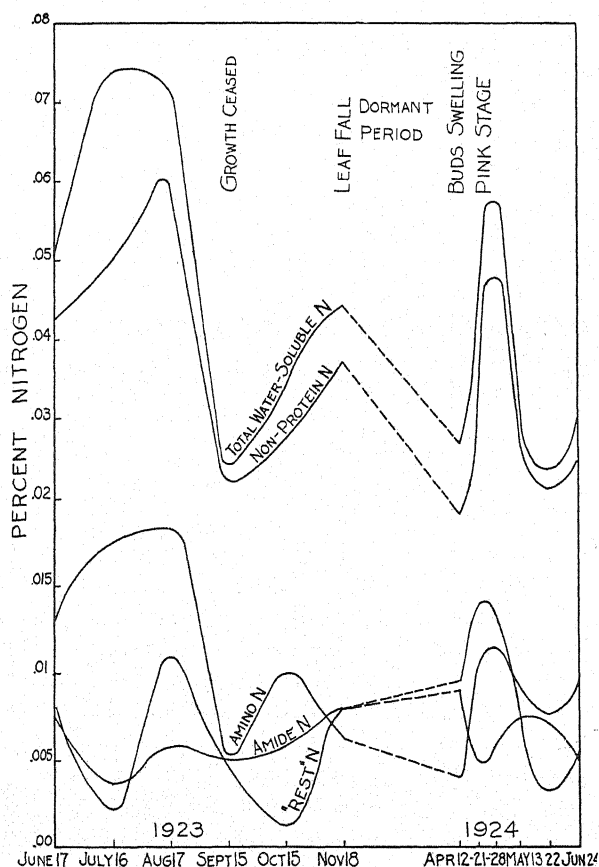


FIG. 4. Total water-soluble N, non-protein N, amino N, amide N, and "rest" N of one-year branch growth as percentages of the fresh weight.

pointed out by VICKERY (43), the presence of a number of other amino-acids found by him in appreciable amounts in alfalfa sap may indicate that the translocating processes are not so simple as that indicated by CHIBNALL and PRIANISCHNIKOW. It is expected that some additional light will be thrown on this subject later in the present series of investigation on *Pyrus malus*.

The sharp decrease in total nitrogen occurring in the one- and two-year branch growth and non-bearing spurs, between the beginning of vegetative growth and the period of blossoming, is accompanied by a rapid decrease in the total water-soluble, non-protein, and free amino nitrogen, but a gradual increase in the amide N. This seems to indicate that the material for the development of flowers was being drawn rapidly from the "reserve" proteins in the form of amides.

During the period of active growth, the water-soluble non-protein nitrogen, including the amino N, again increases. The fact that the amide N simultaneously decreases may indicate its connection with the utilization of amide N in protein synthesis. At times throughout the cycle the amide and amino N show inverse relationships. Since there is evidence (34) to indicate that in the germination of seeds amide N is formed from amino N, some such relationship may hold throughout in these experiments but may be masked at times by the difficulties involved in drawing sharp distinction between the groups. When active growth is over both the total water-soluble nitrogen and the non-protein nitrogen, including the amino and amide N, decline rapidly until the fall evacuation from the leaves commences. During this process of migration of nitrogen both the water-soluble and non-protein nitrogen increase up to the last sampling (November 20). The course of the fluctuation of the non-bearing spurs follows that of the one- and two-year branch growth.

During the so-called "dormant" period the amide N has increased but the amino N has decreased. A small amount (0.13 gm.) of a substance corresponding to the properties of asparagine was isolated from the 1923 dormant branch growth by a method to be given in a later paper.

Since asparagine contains both the $-\text{CO} \cdot \text{NH}_2$ and $-\text{NH}_2$ groups, the latter in the α -position, it is clear that other amino-acids are (as would be expected) present; otherwise the amide and amino N would always parallel one another throughout the cycle. In a later investigation an attempt will be made to identify the amines and amides present.

Summary

The quantitative changes of the principal nitrogen fractions in the non-bearing spurs and in the one- and two-year branch growths and leaves may be summarized:

I. PERIOD OF BUD SWELLING

(a) *Branches and non-bearing spurs.*—Total nitrogen, total water-soluble nitrogen, non-protein nitrogen, amino N and "rest" N increase; amide N decreases.

II. PERIOD OF COMMENCEMENT OF VEGETATIVE GROWTH

(a) *Branches and non-bearing spurs.*—Total nitrogen, total water-soluble nitrogen, non-protein nitrogen and amino N decrease; amide N increases.

III. PERIOD OF ACTIVE GROWTH

(a) *Branches and non-bearing spurs.*—Total nitrogen, total water-soluble nitrogen, and amino N increase; amide N decreases.

IV. PERIOD OF CESSATION OF ACTIVE GROWTH

(a) *Branches and spurs.*—Total nitrogen, total water-soluble nitrogen, non-protein nitrogen, amino N and "rest" N decrease; amide N increases.

(b) *Leaves.*—For the first period to the middle of August, the total nitrogen, total water-soluble nitrogen, non-protein nitrogen, amino N and amide N decreases; the "rest" N remains unchanged. During the second period to the middle of September, all the aforementioned fractions increase except the "rest" N which decreases.

V. PERIOD OF CHLOROPHYLL DEGENERATION

(a) *Branches and non-bearing spurs.*—Total nitrogen, total water-soluble nitrogen, non-protein nitrogen and amide N increase; amino N decreases.

(b) *Leaves.*—Total nitrogen, total water-soluble nitrogen, non-protein nitrogen and amino N decrease; amide N and "rest" N increase.

From these variations certain specific facts showing some of the more definite relationships have been discussed. These are:

1. The total nitrogen and the various partition fractions of the leaves vary with growth, decreasing when growth is very rapid. The total water-soluble nitrogen, the non-protein nitrogen and the amino N parallel the total nitrogen throughout the cycle; the amide N and the "rest" N tend to vary inversely as these fractions. Since the total nitrogen parallels the insoluble nitrogen and since the latter consists chiefly of the insoluble cytoplasmic protein (33), this may be regarded as supporting CHIBNALL'S contention (3) that amino N is connected with protein synthesis and the "rest" N with protein degradation.

2. The total nitrogen and the partition groups of the "wood" growth (spurs and branches) also vary with growth, increasing when it is rapid.

The total nitrogen decreases rapidly from the period of bud swelling to the period of full bloom, and the amount of the decrease is indicative of the demands of the young shoots for nitrogen stored in the phloem.

3. The indications are that during bud swelling the reserve proteins are transported to the actively growing parts in the form of amino-acids.

4. There can be no question as to the phenomenon of autumnal migration of nitrogen from the leaves to the branches in this species. Storage is mainly in the one- and two-year growths.

5. In the aerial parts nitric N (nitrates) was found only at one period, *viz.*, in the buds just as they were opening.

6. In this species nitrates are transformed to amino-acids for the most part in the fine roots.

7. The soluble protein nitrogen is very small throughout the cycle.

8. Ammonia nitrogen is very low throughout the cycle.

9. Nitrogen equilibrium is just about maintained by the application of 5 lbs. of NaNO_3 to a 15-year-old Stayman Winesap tree growing in sod.

10. The importance of the possible rôle by the "rest" N compounds is pointed out. The results tend to confirm CHIBNALL's theory that they are connected with protein degradation.

11. Owing to the small quantities not only of soluble proteins but also of the total water-soluble nitrogenous products, this species is not suitable for investigations on the mechanism of protein synthesis.

The writer desires to thank Professors C. A. SHULL and H. W. POPP for suggestions and criticisms relating to the manuscript and to acknowledge the assistance of Mr. C. A. KERN in the determination of the imbibitional and hygroscopic water, and of Miss ETHEL GINGRICH in the computations of the analytical data.

DEPARTMENT OF AGRICULTURAL AND BIOLOGICAL CHEMISTRY,
AGRICULTURAL EXPERIMENT STATION,
THE PENNSYLVANIA STATE COLLEGE

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THE CURRENT MINERAL NUTRIENT CONTENT OF THE PLANT SOLUTION AS AN INDEX OF METABOLIC LIMITING CONDITIONS*

B. E. GILBERT, F. T. MCLEAN AND W. L. ADAMS

(WITH SEVEN FIGURES)

Introduction

The rate of metabolism at any time during the life of a plant is usually governed by one or more limiting conditions. These conditions limiting metabolism may be either chemical or physical in nature; either a limiting supply of raw materials for metabolism or some condition due to the physical state of the soil, or atmosphere which interferes with the assimilation and elaboration of these raw materials. In this paper attention is drawn to three cases of inhibited metabolism and the accompanying effects on the current mineral nutrient content of the plant solution during 1926. The conditions which were seemingly responsible for decreased metabolism were:

1. A decreased supply of available manganese in a neutral soil, resulting in a marked chlorosis in beets and spinach.
2. Limiting amounts of phosphoric acid and nitrogen as supplied to the soil in fertilizers.
3. Unfavorable weather or cultural conditions.

Analytical methods

The methods discussed by GILBERT in an earlier publication (2) were followed with certain changes of procedure. It was found that when the plant solution was heated to 80° C. before filtering, flocculation took place and filtration was facilitated. Further work with the colorimetric method for potassium led to the adoption of the LINDO-GLADDING gravimetric method. This change was made due to the labor and time involved in recovering the waste platinum from the colorimetric method and also from the fact that by the gravimetric method it was found possible to determine accurately the amounts of potassium usually found in the plant solution.

A limiting available manganese supply

For many years the Rhode Island experimental plats have grown crops which from time to time were distinctly chlorotic. It has been found that

* Contribution no. 345 of the Rhode Island Agricultural Experiment Station, Kingston, R. I.

this chlorosis occurs only with plants growing on soils which have been neutralized, and recently the correction of the conditions has been brought about by small applications of manganese salts in a spray (3), or as a fertilizer mixed with soil. Chemical determinations of the leafy tissue of crop plants have shown smaller amounts of total manganese in the chlorotic tissue than in the normal healthy green tissue (3). These results have led investigators to conceive the available manganese supply in the neutral or alkaline soil to be limiting and insufficient for normal metabolism. This condition proved to be most pronounced when coupled with a low nitrogen fertilization.

Plant solution determinations were made to compare the nutrient element levels in the chlorotic and non-chlorotic plants. In table I a comparison is given of the manganese fertilization and the nitrate-nitrogen as found in the plant solution.

TABLE I

COMPARISON OF NITRATE-N CONTENT OF PLANT SOLUTION AND MANGANESE FERTILIZATION

CROP	TISSUE	PLAT	FERTILIZATION (LBS. PER ACRE)				CONDI- TION OF PLANTS	NITRATE-N IN PLANT SOLUTION P. P. M.		
			N	P ₂ O ₅	K ₂ O	Mn.				
Spinach	Leafy	21 MG*	0	200	30	11	Green Chlorotic Green	June 2		
Spinach	Leafy	21 MG	0	200	30	0		50		
Spinach	Leafy	50 MG	60	200	30	9		283		
Beets	Leaves and petioles	119 MG	45	120	90	9	Green	Sept. 17	Oct. 1	Oct. 8
	Leaves and petioles	119 MG	45	120	90	0	Chlorotic	59	42	30
Beets	Roots	119 MG	45	120	90	9	Green	176	104	28
Beets	Roots	119 MG	45	120	90	0	Chlorotic	200	283	125
Beets	Roots	118 MG	90	120	90	9	Green	600	625	213
								277		

* Market Garden Plat.

The application of manganese salts was accompanied by an increased yield as noted with previous crops (3) and also a decreased reserve of un-metabolized nitrogen as nitrate. The potassium reserve was likewise much reduced in the greener plants of spinach grown on plat 21. The manganese-treated plants contained potassium to the extent of 768 parts per million, while those from the untreated area showed 1,213 parts per million in the plant solution.

Considering the nitrogen situation with spinach on plat 21, it must be noted that in reality there were two limiting fertilizer conditions operating, *viz.*, insufficient manganese supply and a diminished nitrogen supply. This is seen when a comparison of yields of spinach is made between the manganese-treated areas on plat 21 and plat 50 (near plat 21 and had received nitrogen fertilization of 60 pounds per acre). Plat 21 was depressed 59 per cent. as compared to plat 50. This fact may help to account for the magnitude of the difference between the nitrate-nitrogen content of the plant solution on the treated and untreated areas of plat 21, the limiting nitrogen supply having accentuated the condition.

When beets growing on plat 119 are considered, however, nitrogen having been included in the fertilization, a comparison of yields between treated areas of plat 119 and plat 118 (adjacent and fertilized with 90 pounds of nitrogen per acre), shows the decrease of yield with decreased nitrogen to be only 26 per cent. Hence nitrogen as a limiting factor was less active. The nitrate-nitrogen difference between the treated and untreated beet plants is thus due more directly to the limiting manganese supply. The accumulation of nitrates both in the petioles and in the storage roots of beets with deficient manganese supply, is fully as marked as in the leaves of spinach. Only at the end of the growing season, on October 8, did the nitrogen content of the leaf petioles become equalized in the chlorotic, manganese-deficient plants and in the normal ones. This may be accounted for by the withdrawal of nitrogen from the old leaves, as there was still a relatively high nitrate content in the roots of the chlorotic plants on that date.

Insufficient nutrients as metabolic limiting conditions

The most obvious conditions instrumental in limiting metabolism, are insufficient supplies of raw materials. Under surroundings such as occur in nature these are usually considered to arise from deficiency in or unavailability of the supplies of nitrogen, phosphoric acid, and potassium, as found in the soil in which the plant grows. When any one of these nutrients becomes limiting in supply, the limitation is reflected in the growth and may be measured by the yield at the end of the growth period. If, due to some such limiting nutrient supply a difference in growth or yield results, other conditions being the same, some reflection may be expected in the amounts of this nutrient in the plant solution. It may also be profitable to examine the relationship of another nutrient to the limiting one, as shown by the plant solution content.

In table II some data are given which show clearly the reflection in the plant solution of the amounts of nitrogen and phosphoric acid applied in fertilizer chemicals. An increase in either nutrient is shown in the current amounts of nitrate-nitrogen and phosphate-phosphorus.

TABLE II

COMPARISON OF NITRATE-N AND PHOSPHATE-P IN PLANT SOLUTION WITH LIMITING SUPPLIES OF NUTRIENT APPLIED TO SOIL*

CROP	TISSUE	PLAT	RELATIVE YIELD OF TISSUE	NITRATE-N IN PLANT SOLUTION P. P. M.				PHOSPHATE-P IN PLANT SOLUTION P. P. M.					
				July 20	Aug. 3	Aug. 16	Sept. 1	Sept. 14	July 20	Aug. 3	Aug. 16	Sept. 1	Sept. 14
Turnip	Roots	55N	95	681	614	506	545	189	9.0	3.0	2.00	5.4	9.6
Turnip	Roots	65N	100	535	545	580	268	395	50.0	34.0	13.8	20.8	11.7
Turnip	Roots	65S	95	625	652	567	380	428	20.0	37.5	13.5	13.0	6.6
Corn	One foot of stem above highest node												
Corn	One foot of stem above highest node	55N	92	July 16 340	July 28 319	Aug. 9 113			July 16 33.3	July 28 26.8	Aug. 9 1.00		
Corn	One foot of stem above highest node	65N	100	319	288	79			38.4	28.8	5.7		
Corn	One foot of stem above highest node	65S	100	405	394	102			40.0	37.5	6.9		
Cabbage	Lower leaves minus midribs												
Cabbage	Lower leaves minus midribs	55N	54	Aug. 20 250	Aug. 31 155	Sept. 13 97			Aug. 20 0.86	Aug. 31 4.45	Sept. 13 1.38		
Cabbage	Lower leaves minus midribs	65N	94	256	169	40			1.11	5.20	1.56		
Cabbage	Lower leaves minus midribs	65S	100	325	187	88			1.18	1.15	0.75		
Carrots	Roots												
Carrots	Roots	55N	97	July 27 147	Aug. 5 214	Aug. 18 102	Sept. 3 49	Sept. 16 25	July 27 25.0	Aug. 5 13.4	Aug. 18 18.0	Sept. 3 17.0	Sept. 16 11.7
Carrots	Roots	65N	100	100	137	69	71	76	31.0	15.5	19.7	21.5	14.7
Carrots	Roots	65S	93	78	318	91	169	31	31.0	15.5	19.7	16.5	10.8

* Fertilizers applied as N: P₂O₅: K₂O: Plat 55 North 55: 50: 135 lbs. per acre.
65 North 55: 150: 135 lbs. per acre.
65 South 80: 150: 135 lbs. per acre.

The story as regards relative yields as measures of growth is, however, not so clear, and needs more explanation than is given in the table. With all the crops tested the increase in phosphoric acid as supplied between plats 55N and 65N is clearly shown both by the phosphate-phosphorus in the plant solution and by the relative yields. But when we compare relative yields of tissue of plats 65N and 65S, the latter of which received 25 pounds extra nitrogen per acre, we find that with turnips and carrots a decrease in yield was obtained in the case of 65S; with corn no difference resulted; while with cabbage there was an increase of 6 per cent. in the relative yields. If the particular tissue examined be considered, this variation in yield will be understood. With turnips and carrots the root tissue was analyzed while in the case of cabbage the leaves were used for analysis. The relative yields given in the table are of these tissues. If, however, the weights of turnip tops from plats 65N and 65S be expressed as relative yields they are 81 and 100 respectively. Thus the additional nitrogen fertilizer tended to produce leafy growth at the expense of the other portions of the plants. Nitrogen was limiting for leafy growth only, and as the tissue examined in the case of cabbage was leafy, this was reflected in the yield. With corn the stem tissue examined may be considered to have been intermediate and not likely to be greatly affected by the extra nitrogen of 65S.

Considering next the effect of the supply of one nutrient upon the plant solution content of another, we find that when the phosphoric acid applied and the phosphate-phosphorus are low, the nitrate-nitrogen in the plant solution is high and *vice versa*. This seems to hold with turnips, corn and carrots, but not with cabbage. In this latter crop, however, the phosphate-phosphorus differences between the plant solutions of plats 55N and 65N were small and may not have been great enough to affect the nitrate-nitrogen content. The reciprocal story is, however, not so clear. With the extra nitrogen applied and the higher nitrate-nitrogen content of the solution in the case of 65S, the phosphate-phosphorus was lower with turnips and cabbage only. The story is confused with the other two crops. It would be of interest to observe the effect of a larger increase in nitrogen upon the phosphate-phosphorus content of the plant solution.

Changes in external conditions affecting metabolism

From the foregoing facts it is evident that differences in the nutrient level are reflected in the composition of the plant solution. There are also temporary changes in the concentrations of fertilizer elements in the plants. When such changes simultaneously affect the composition of the plants growing under a variety of conditions of soil fertility, then we may expect to find that they are due to some external condition. Therefore, a study was made

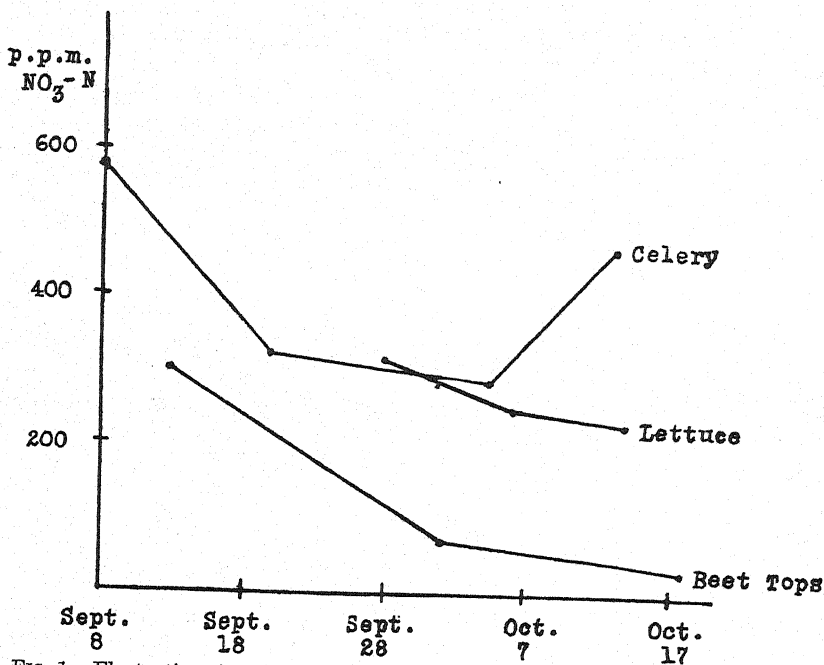


FIG. 1. Fluctuations in nitrate-nitrogen during decreasing temperature conditions.

of the weather conditions, and of the temperature and moisture content of the soil, to see if these factors could be correlated with changes in concentrations of the plant solution.

A conspicuous instance of this occurred about October 4 when the plant solution concentrations were very low. In figs. 1, 2, and 3 are shown graphical representations of the nitrate-nitrogen, phosphate-phosphorus, and potassium concentrations found at that time in celery, lettuce, and beet tops. The graph for each crop represents an average of determinations from two or more plats where nutrient levels were not limiting. It will be noted that with the exception of phosphorus in beet tops the three nutrient elements decrease in concentration through the month of September, and reach about a minimal value around October 4 for all nutrient elements and crops. A marked exception to this tendency for lowered concentration was noted in the case of late spinach which was planted on August 27 and showed a high nitrogen content on September 27.

The diminishing concentration of the plant solution of the crops was accompanied by a decreasing amount of nitrate-nitrogen in the soil.*

Except where large amounts of nitrogen were added during the month,

* Determinations by J. B. Smith, Assoc. Chemist.

TABLE III

NITRATE-NITROGEN CONTENT OF THE TOP 7 INCHES OF SOIL UNDER GROWING CROPS (P. P. M.)

CROP	SPINACH		BEETS		CELERY					
Plat No.	112	113	118	119	85	115	116	58	29	60
Aug. 31	13	10	8	12	4	25	10	13
Sept. 15	61	26
Sept. 27	44	22	8	4	6	28 ^a	5	38 ^a	10	18 ^a
Oct. 8	34	8	8	2	8	8	2	30	8	7

^a Forty pounds of nitrogen per acre in nitrate of soda were added to each of these plats on September 24, three days before sampling. This quite probably increased the nitrate content of the soil in these plats on September 27.

the nitrate content of the soil was low, or at least decreased, from August 31 until October 8, under the crops of spinach, beets, and celery. Plat 112 of spinach and plat 58 of celery were the only ones which retained a high nitrate content through the month of September.

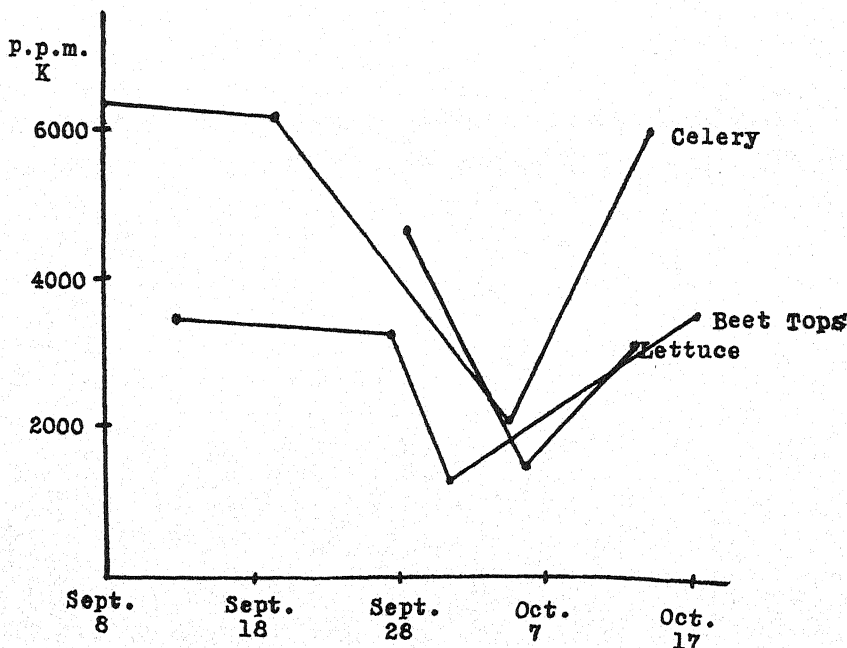


FIG. 2. Fluctuations in potassium during decreasing temperature conditions.

During the month of October the concentrations of the nutrient elements generally increased in the plant solutions of the three crops (figs. 1, 2, and 3).

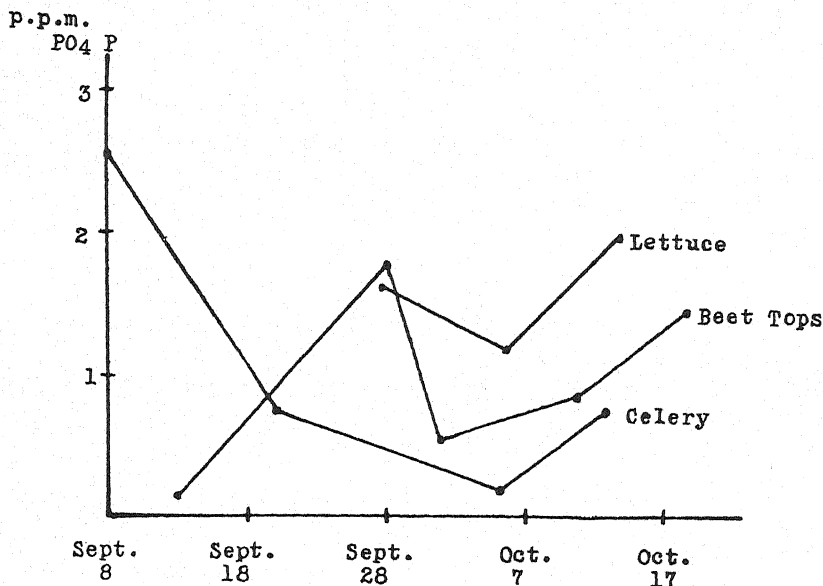


FIG. 3. Fluctuations in phosphate-phosphorus during decreasing temperature conditions.

These were accompanied by a marked decrease in both soil and air temperatures, as shown in fig. 7. This fall in temperature may have contributed to the accumulation of inorganic nitrogen, phosphorus, and potassium compounds in the plants, by depressing growth and decreasing the rate of metabolism.

The growth rates of the different crops studied were quite uniform throughout the period of most active growth, passing through a regular grand period of growth for each crop (figs. 5 and 6). Changes in weather and other changes of temporary character in the external conditions seemed to have little effect on the growth rate. The growth of every crop was markedly depressed by deficiencies of N, P, and K on plats which were purposely kept deficient in these fertilizer elements.

The only noticeable depression in growth increments which may be attributed to temporary unfavorable conditions for growth was during the period from July 15 to July 29. This showed as a depression in the growth of cabbage leaves as measured on July 22 (fig. 5). The index of leaf area used here in measuring the leaf growth of cabbage was the leaf product, obtained by multiplying the greatest width by the greatest length. This gave a value much larger than the actual leaf surface, but is believed to be approximately proportional to the leaf area (4), (5), (6).

There was a depression in height growth of turnips also, noted on July 29. Height of turnips was measured from the ground surface to the tip of the tallest leaf.

These two depressions in growth were correlated with notably high values for the nitrate content of the plant solutions; of cabbage on July 29, and of turnips and carrots on August 6 (fig. 4). This depressed growth of the plants and attendant accumulation of nitrate in the plant solution was accompanied by and followed a period of hot weather extending from July 15 to 25 (fig. 7).

The moisture content of the soil was not critically low at any time during the summer. It fluctuated between 20 per cent. and 35 per cent. of the dry weight of the soil. The moisture-holding capacity of these soils by the HILGARD test was from 36 per cent. for the mineral soils to 44 per cent. for the heavily-manured soils on the Market-Garden plats. Thus the calculated wilting coefficient of the soil by the BRIGGS and SHANTZ formula (1) was

p.p.m.

NO_3N

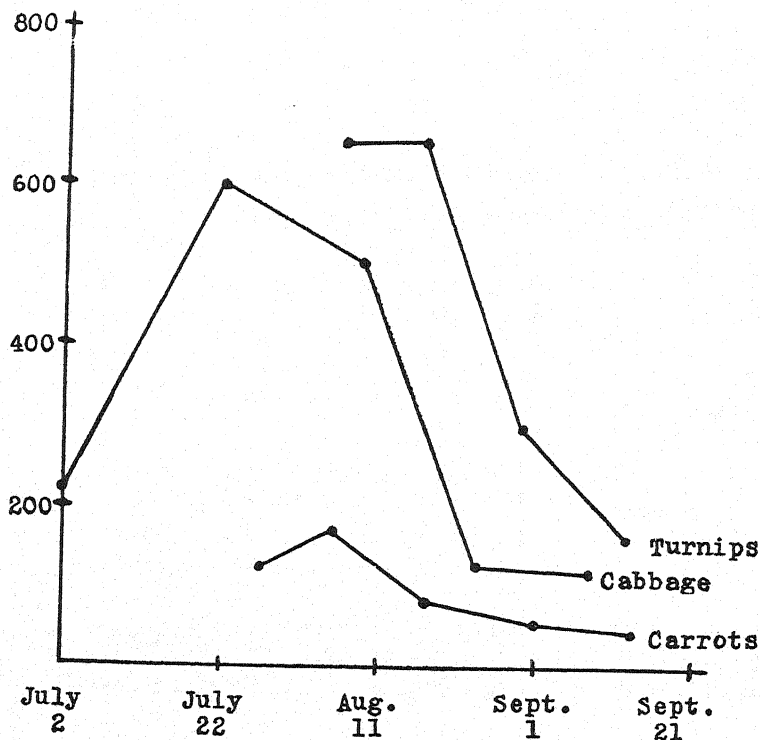


FIG. 4. Accumulation of nitrate-nitrogen during high temperature period, July 15-July 29.

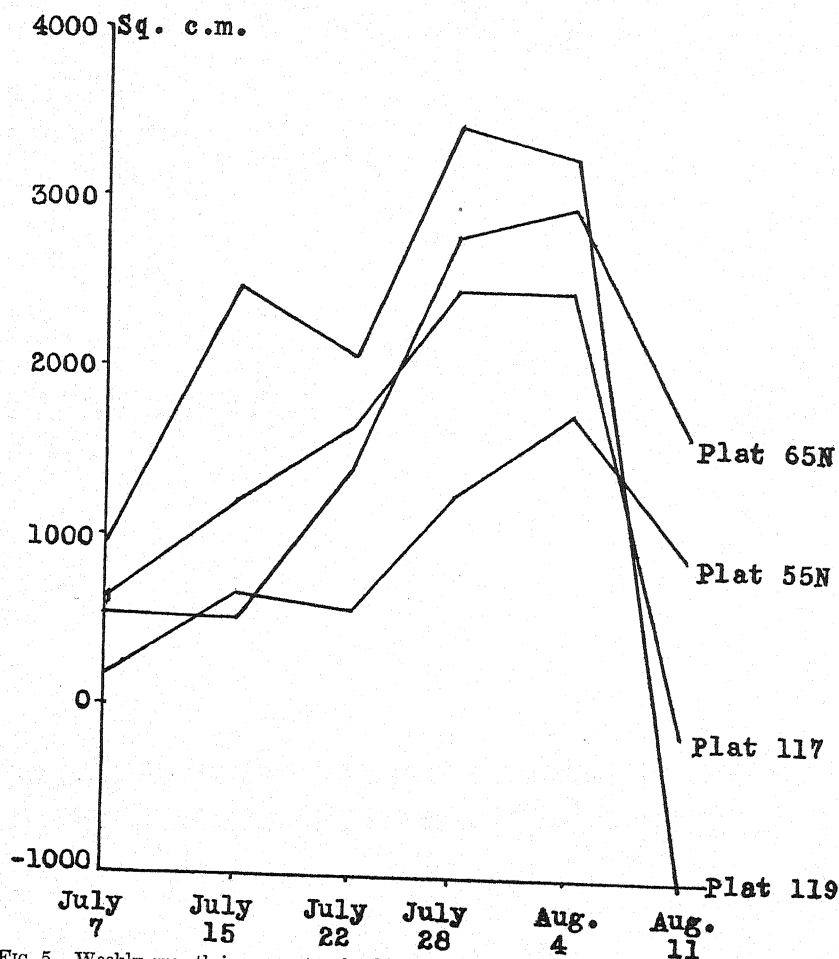


Fig. 5. Weekly growth increments of cabbage on well fertilized Experiment Station plats, 1926. Leaf product (length \times width).

about 12 per cent., and the usually accepted optimum moisture content of the soil for plant growth—50 per cent. of the water-holding capacity—was approximately 18 per cent. to 22 per cent. So the moisture content of the soil does not appear to have been at all critically deficient at any time during the growing season.

Discussion

The current mineral content of the plant solution has been shown to be an index of metabolic limiting conditions in three cases of inhibited

metabolism and these three cases have been discussed in turn. There now remains the developing of a concept to embrace limiting conditions in general. The authors have found it convenient to contrast the individual nutrient concentration in the plant solution with the level of water in the reservoir of a city supply system. This level is determined by the rate of inflow and the demands made upon the system by water consumption. In the plant, the concentration of any one nutrient element is determined by the available supply of that element in the soil and the permeability of plant membranes to the nutrient element. These govern the rates of inflow. At the other end of the system, use of the individual nutrient element is determined by the rates of metabolic processes, which are subject to changes through the action of limiting conditions. A further factor which may be expected to affect the concentrations is the amount of moisture present in the plant tissues. As this decreases with increasing saturation deficit, the plant solution may be expected to become proportionately more concen-

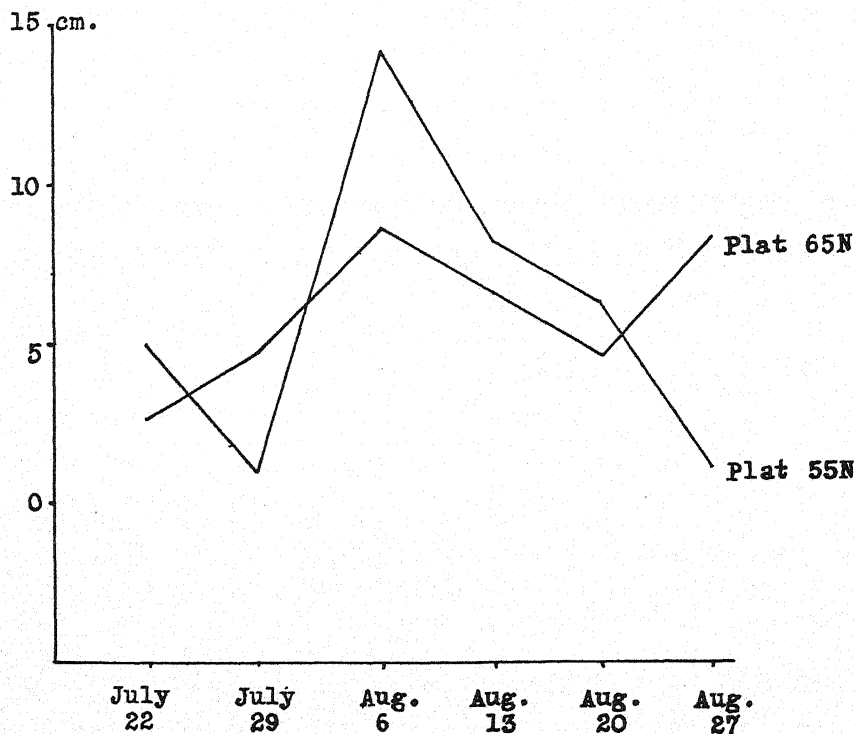


FIG. 6. Weekly growth increments of turnips on well fertilized Experiment Station plots, 1926. Height of tallest leaf in cm.

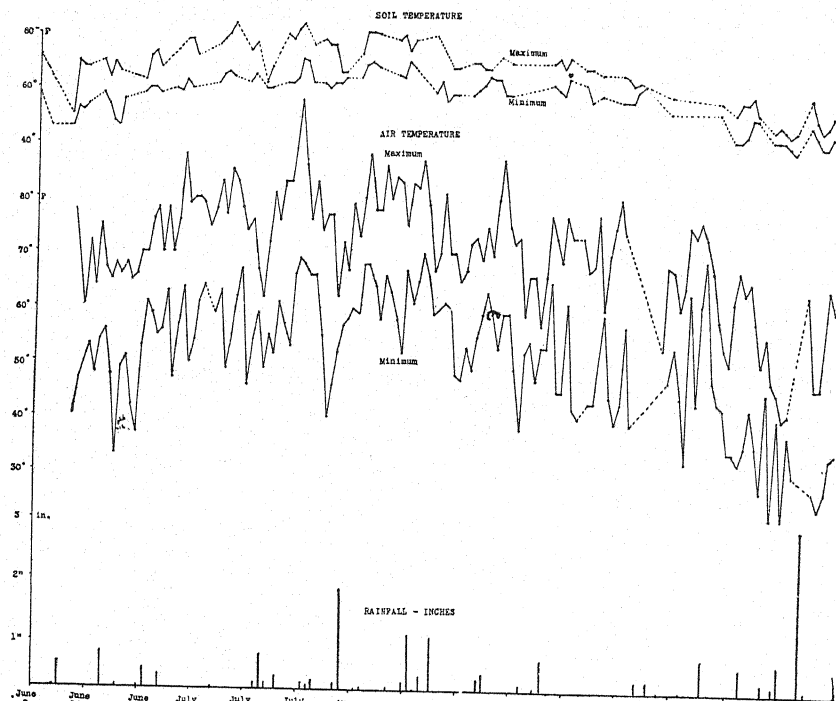


FIG. 7. Weather observations at Experiment Station plats during the growing season of 1926.

trated. The extent of this effect has not been determined as yet, but it may be expected to be small except under severe conditions of evaporation, when it also would assume the rôle of a limiting condition and be reflected in the current nutrient-element concentration.

Summary

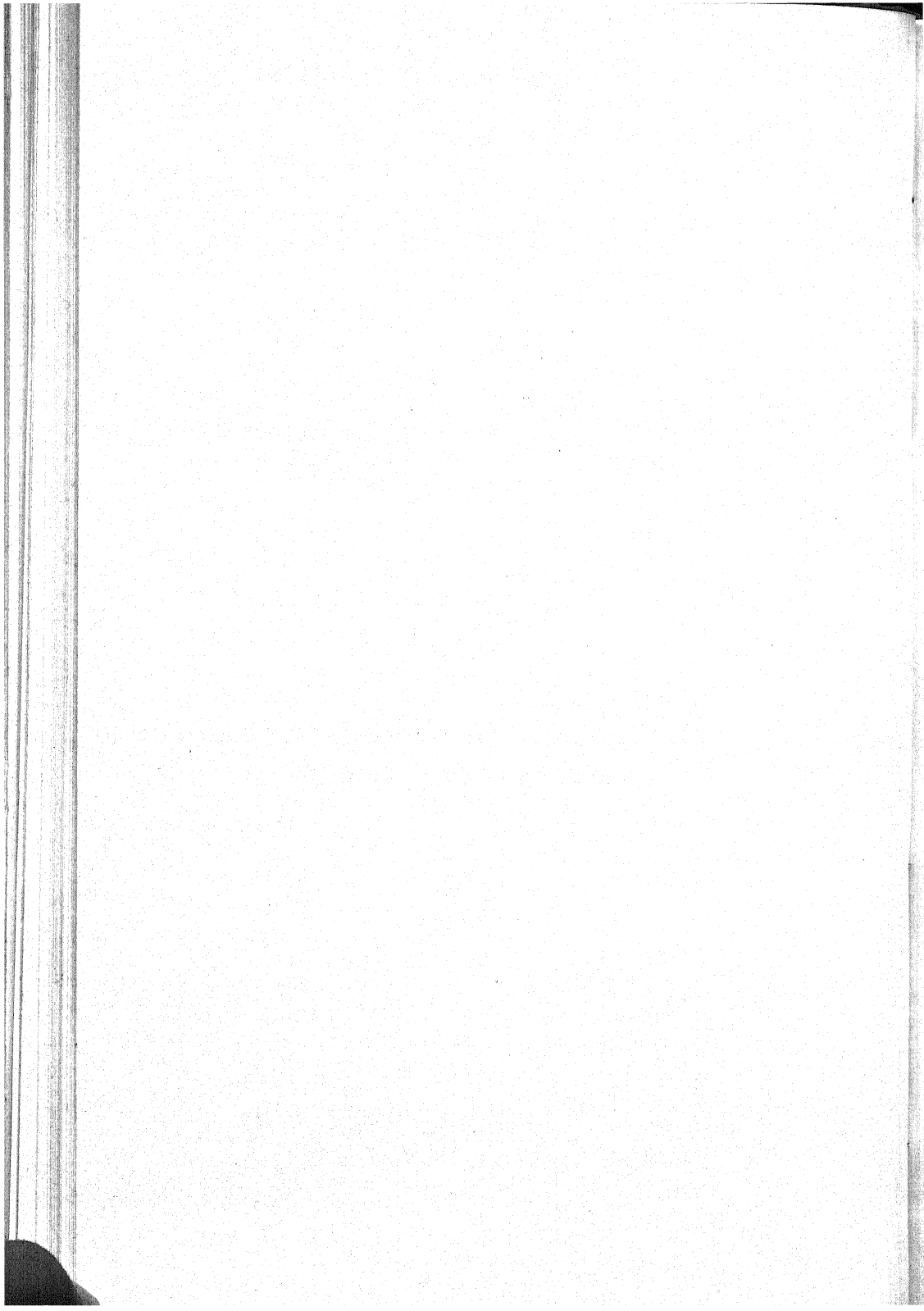
In this paper the current amounts of nitrate-nitrogen, phosphate-phosphorus, and potassium are shown to have been correlated with arrested metabolism which was induced by the following limiting conditions:

1. A decreased supply of available manganese in a neutral soil, resulting in a marked chlorosis in beets and spinach.
2. Limiting amounts of phosphoric acid and nitrogen as supplied to the soil in fertilizers.
3. Unfavorable weather and cultural conditions.

RHODE ISLAND AGRICULTURAL EXPERIMENT STATION,
KINGSTON, R. I.

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GROWTH STUDIES ON FRUITS

AN EXPLANATION OF THE SHAPE OF THE GROWTH CURVE*

F. G. GUSTAFSON

(WITH THREE FIGURES)

In a recent paper the writer (6) has shown that the growth curves of fruits are similar to those of animals and vegetative parts of plants. This has also been shown by ANDERSON (1) and GOLINSKA (4). Some of the data used in the previous paper were obtained from fruits set at different times and it was suggested that it might be more accurate to have a large number of plants in blossom at the same time and on these tag several thousand blossoms growing in identical positions on the plants. Collections for volume and weight determinations could be made from these every week or more often if desired.

This was done during the summer of 1926. Figs. 1 and 2 show the result of this experiment. The volume and weight measurements were made every other day for the first three weeks and after that every week. The number of fruits used varied from 200 at the time of setting to 50 from the ninth day to the end. A conscious attempt was made to pick average fruits each time. These fruits were all weighed together and the average individual weight obtained. Their average individual volume was obtained by finding the volume of water displaced by them. The volume and green weight curves are almost identical.

As everyone knows who has studied growth, ROBERTSON (13) and OSTWALD (9) independently were the first to liken growth to an autocatalytic reaction of the first order, which also gives an S-shaped curve. Both of these writers, using their own data as well as that of other investigators, compared the observed growth curves with the calculated curves, using the monomolecular autocatalytic equation. Both report close agreements, as have other investigators since.

Recently, however, GREGORY (5) maintains that only the latter half of the growth curve of barley can be represented by an autocatalytic reaction equation. VAN DE SANDE-BAKHUYZEN (2) states that the calculated and observed values do not agree at all. Thus we see that on the basis of calculation, two investigators have during the last year denied that the ROBERTSON and OSTWALD formula holds for the growth of plants.

* Paper from the Department of Botany of the University of Michigan, no. 256.

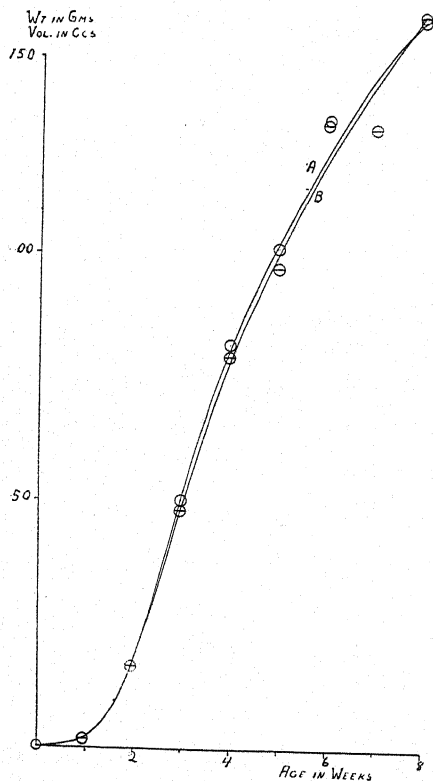


FIG. 1. Growth curves of tomato fruits. Volume and green weight determined weekly, from 200-50 picked fruits, previously tagged at the same time. Curve A, average volume of one fruit; curve B, average green weight of one fruit.

There are many investigators who explain growth in other ways than by autocatalysis. This is particularly true of investigators working with unicellular organisms such as bacteria and yeast. Thus McKENDRICK (7), studying bacteria, came to the conclusion that "The rate of multiplication of fast-growing micro-organisms is proportional to the number of organisms and to the concentration of food stuffs."

SLATOR (15) has found that "If the seeding is small and all necessary food for yeast growth is in excess, the growth during the earlier stages of the reaction is unrestricted and follows the logarithmic law of increase, that is the rate of increase is always proportional to the quantity present. At a later period retarding influences come into action, the yeast multiplication becomes restricted and during the final stages of fermentation ceases entirely."

PRIESTLEY and PEARSALL (12) explain the growth of roots of *Tradescantia zebrina* and *Lycopersicum esculentum* on the basis of food supply. They

noted several S-curves in the total growth of the root system and the flattening out of the curve seemed to be associated with the formation of the secondary or tertiary roots. PEARSALL (10) in a later paper has elaborated the theory that food supply is a factor in the growth curves of roots.

MURNEEK (8) explains the vegetative growth in tomato on the basis of correlation. He believes that the presence of fruits on a vine slows down the growth and finally inhibits the vegetative growth entirely. He shows that by the removal of the fruits the growth does not slow down but continues at the same rate as before.

From my own studies on the fruits of cucumber, summer squash, muskmelon and especially tomato I am inclined to believe that growth of fruits is mainly a matter of nutrition. By nutrition is meant the supply of food, mineral material, water, oxygen and any other material used in building up and maintaining the cells. This is the same idea that PRIESTLEY and PEARSALL have made use of in explaining root growth, applied to the growth of fruits.

If for a moment we go back to SACHS's experiments (14) on growth of roots of *Vicia faba* and the stem of *Phaseolus multiflorus* and plot his data as usual, *i.e.*, total length against time, we will obtain the typical S-shaped curve. In the experiment a disk of the stem or root 1 mm. thick undergoes

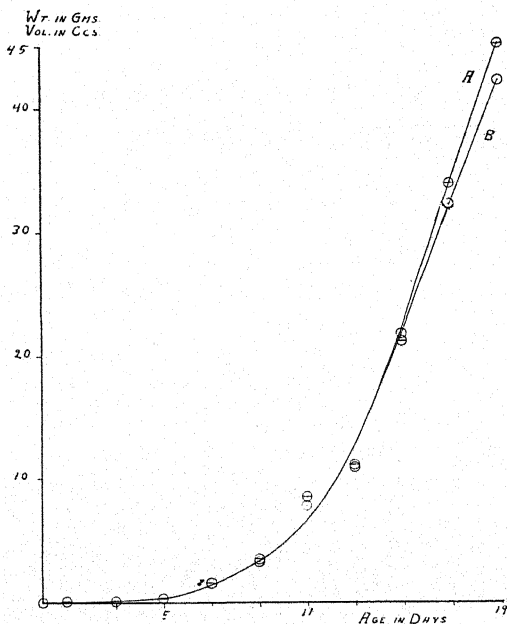


FIG. 2. First 19 days of growth of fruits shown in fig. 1. Measurements made on alternate days. First reading from 200 ovaries; later readings from decreasing numbers, 50 on the ninth day and subsequently. Curve A, volume; curve B, green weight.

a growth cycle which corresponds to that of a whole organism, or an organ of such an organism. This growth is made up of the cell formation (cell division), cell enlargement, and sometimes a further increase in mass brought about by the thickening of the cell walls or deposition of stored food material. In cell division there is very little if any increase in size, because the two cells will occupy the space of the one. However, when the meristematic cell enlarges preparatory to the division there probably is a slight increase. After the new cell has been formed, it begins actively to increase in size due to intake of water. This is at first not very rapid but it becomes more and more so. However, a time comes when not so much water is absorbed due to the fact that the forces which tend to keep the cell from further enlargement are nearly equal to the forces causing the water to enter the cell. When the two are equal there will be no further increase in size of the individual cell and growth as illustrated by increase in volume will cease. After the cell has stopped increasing in size there may of course be a thickening of the cell wall and even a storing of food material so that the mass of the cell would continue to increase for some time after the volume increase ceased. In fruits the increase in volume and dry weight are very closely associated as shown in the previous paper and in fig. 1 of this paper.

The growth of an organism is the sum of the growth of its individual cells. If in unit time, the same number of cells were formed and they increased to the same volume and mass during the whole life of an individual the rate of growth would be constant, but that is not a fact. In the young individual there are few cells either being formed or increasing in volume or mass. Consequently the actual increase in size must be very slow. If each cell kept on dividing at the same rate we would have a geometric rate of growth, which is approximated by the early part of the growth period. This period is followed by another in which the growth is proportional to time, and in turn this period gradually changes into one where there is very little and finally no growth at all. This may be explained by assuming that the number of cells formed gradually becomes smaller and finally ceases altogether or else the final size reached by each cell is less in the later life of an organism than in its early life. There is no evidence for the latter supposition in tomato fruits.

The question may fairly be asked, why does cell formation stop as the organ or organism becomes old and mature? My answer would be that there is a lack of nutrient material, *i.e.*, foods, minerals, water, etc.

For an elaboration of my thesis I wish to use as an illustration the tomato fruit. There is no question but that an available supply of nutrient material influences the size of the fruits to a great extent. It is a well established practice to thin the fruits, when the setting has been very heavy, to

obtain fewer but larger fruits. In the case of tomato it was noted in the paper cited that the first fruits to set on a plant are smaller than those setting later, when the plant is larger and more vigorous. If the plant is prevented from setting fruit until it is large, the first fruits will be large. The second fruit in a fruiting cluster is usually smaller than the first and the third is smaller than the second. Occasionally the second fruit is larger than the first, but this happens only when for some reason or other the first fruit does not begin to grow until the second fruit is of some size. The explanation presumably is that the second fruit got such a start that the main food supply is directed in that direction and it is so to speak on the main line of supply, while the first fruit has been shunted to the side line. If one examines such a cluster, it is at once noticed that the peduncle has straightened out in such a way that the second fruit is actually in a more direct line to the stem than the first fruit, which is now on a side branch.

It has been noted that when the first fruit is removed, the second, if it has not already commenced to ripen, will begin to grow very rapidly and will usually attain the same size as number one. I do not believe this is due to any inhibiting substances, of which we have heard so much, but of which we know so very little, because in the case of fruits setting on a small and young plant the first are always smaller than those setting later when the plant has more food material. If there were inhibitors, the later fruits ought to be smaller than the first, because they would be inhibited by the fruits first formed, while there would be nothing to inhibit the first fruits set except lack of material from which to grow.

If for a moment we assume that food supply is the factor influencing the rate of growth, how can we explain the shape of the growth curve? When the fruit is set there is an abundance of nutrient material for its needs (not much being needed), but it increases in size very slowly, because there are few cells dividing and enlarging. Later, as more cells are produced and begin to enlarge, the increase in size becomes more pronounced. For this rapid growth more nutrient material is needed and a time will come when there is not enough and the growth cannot go on increasing as it has, and we have a decline in rate. The reason the nutrient material becomes the limiting factor is partly due to the fact that there is not enough in the plant for all the fruits as well as for the vegetative growth and perhaps partly due to poor conduction of material to the growing part of the fruits. Consequently there is a decrease in the rate of growth and when total volume is plotted against age we get an S-shaped curve.

It is a well known fact that, if a few fruits are permitted to mature, the individuals will be larger; yet they will not grow indefinitely, but will eventually reach a limit beyond which they cannot be made to grow, even if only one fruit is produced by the plant. An explanation for the limit

of the size to which a fruit can grow, I believe, is connected with the conduction of materials into the fruit. The conductive system of a fruit like the tomato does not enlarge in proportion to the pulpy part. In the young fruit or in the ovary the vascular strands are fairly numerous and well developed, but the mature or nearly mature fruit is poorly supplied with such structures. For this reason, together with the fact that as the fruit becomes larger the amount of nutrient material needed also increases, it seems reasonable to assume that the conducting system should become inadequate. In the large fruits the food, water, oxygen and mineral material all have to be conducted by diffusion for much longer distances than in the young fruits. Even though the nutrient gradient may be quite high the difficulty of transportation is so great that eventually the fruit stops increasing in size entirely. It is to be remembered that even though the fruit does not increase in volume or mass, yet material must be taken into it unless it is actually to decrease in size. This is true of water at least. Muskmelons and summer squashes growing in bright sunlight and in a dry field may actually decrease in volume considerably while maturing.

The growth of the shoot of a plant can also be explained on the basis of nutrition. When a seed germinates it is supplied with an abundance of nutrient material, but the cells growing are few, and the increase in mass of the plant is very slow at first. In fact, as BRENCHELY (3) has shown, and as found by the author in experiments not yet published, there is in some plants an actual decrease in dry weight for the first two or three weeks, even though the volume increases very extensively. As time progresses, the number of cells growing increases, and the food supply is augmented by the photosynthesis of the leaves. However, not all cells formed become part of the manufacturing tissue nor of the absorptive tissue. For every cell that goes to form chlorenchyma or root tissue there are others that go to form stem, veins in the leaf, and later to form the flowers and the fruits. Yet all of these cells depend upon the cells of the chlorenchyma and of root tissues for their nutrient material. Thus the proportion of nutrient consuming tissue to the nutrient obtaining tissue increases as the plant ages.

MURNEEK (8) has shown that when fruits of the tomato are prevented from forming, the vegetative part of the tomato plant keeps on growing at a more or less constant rate. POPP (11) has also noted that when Biloxi soy beans grown under artificial light conditions are prevented from blossoming the plants keep on growing and the curves obtained are not of the sigmoid type.

The writer conducted an experiment with a variety of pole bean in which a part of the plants were deflorated while other plants were permitted to blossom and fruit. The seeds were planted May 24, and the first measurements were made two weeks later. From that time, measurements were

made weekly except for the tenth week. The weather was very dry during the first eight weeks and the last three of those weeks were also very hot. This probably accounts for the very slow growth during that period. On July 27, nine weeks after planting, the plants were divided into two groups. From that time one group had all the blossoms or flower buds removed as soon as they appeared in an endeavor to prevent any fruits from forming; the other group formed fruits in the usual way. Fig. 3 shows that the

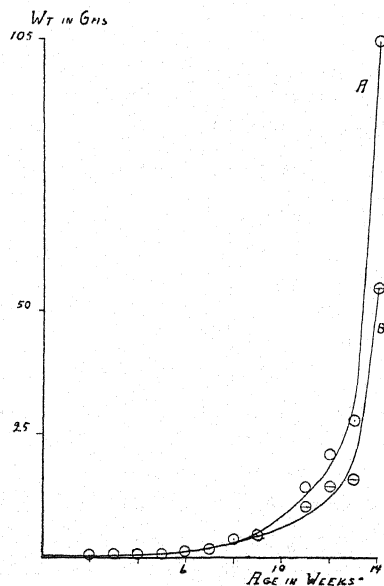


FIG. 3. Curve of increased dry weight for pole bean, in two groups after the ninth week. Curve A, deflorated plants; curve B, fruiting plants. Average final dry weight of plants on curve A, 105.21 gm.; curve B, 55.48 gm.

plants which did not fruit formed a much greater amount of dry material than those which fruited. Not enough plants were available to carry on the experiment till the plants with fruits stopped growing; but as the point of the experiment was to show that when fruits did not have to be supported by the plant the vegetative portion would grow much longer, it was conducted far enough to demonstrate this point clearly.

These illustrations show that when the nutrient material was not diverted from the shoot the total size and dry weight increased much more than when part of the nutrient material was diverted to fruits and flowers. One explanation for this would be that more nutrient material was available for the formation of new leaves, which in turn would increase the dry weight of the plant by photosynthesis.

Summary

1. This paper discusses in some detail the probable influence of nutrition upon the form of the growth curve of fruits. It is also pointed out that the poorly developed conductive system in fruits may very materially limit the nutrient material supplied to the fruits.

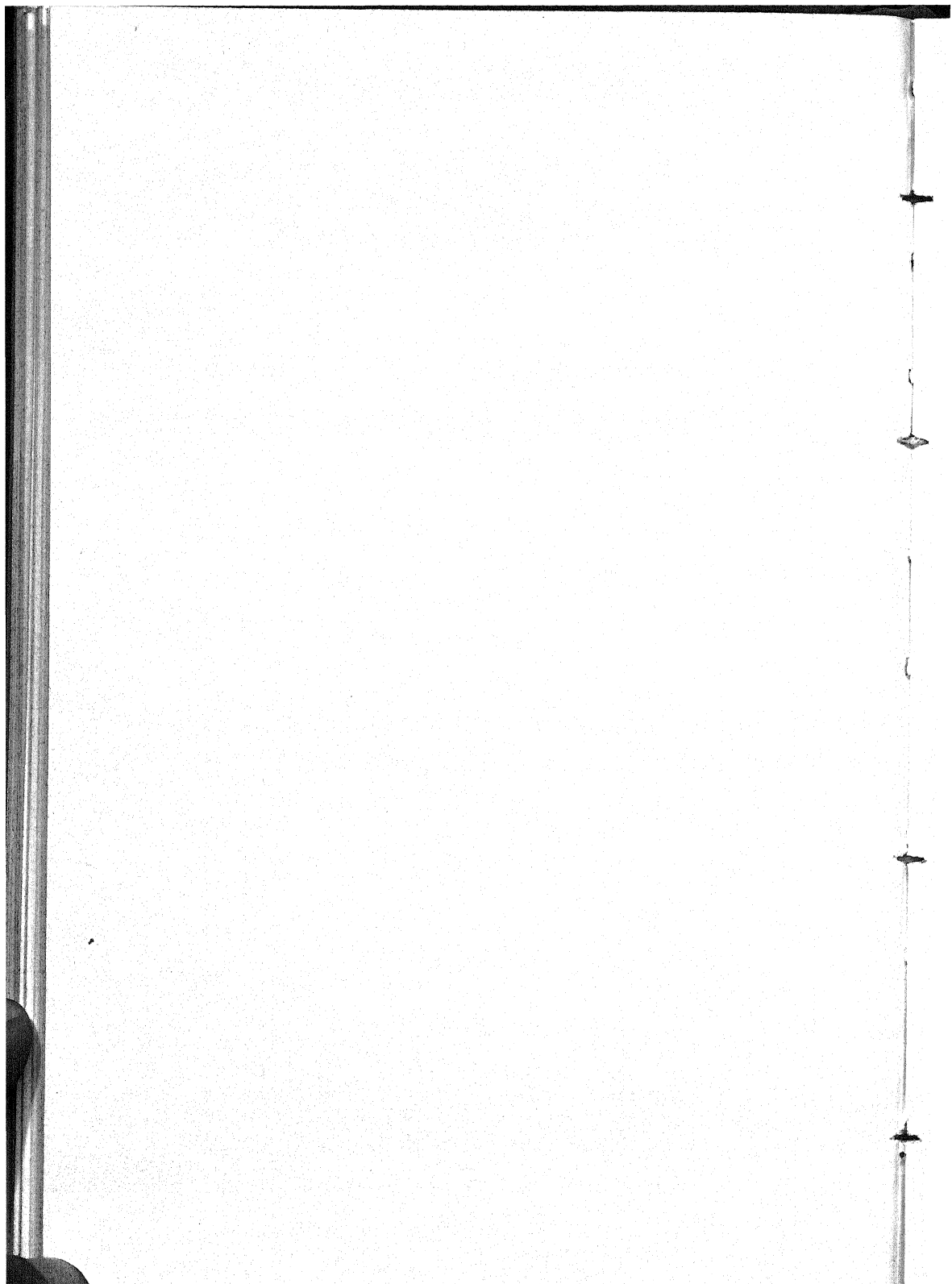
2. It is further pointed out that when nutrient materials are not diverted to fruits, the rate of growth of the shoot continues at an approximately uniform rate, rather than at an increasingly lower rate as is usual when fruiting takes place.

UNIVERSITY OF MICHIGAN,
ANN ARBOR, MICHIGAN

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CHEMICAL CHANGES ACCOMPANYING THE WESTERN YELLOW BLIGHT OF TOMATO*

J. T. ROSA¹

The disease of the tomato plant called Western Yellow Blight is frequently very destructive in western North America but is not known to occur elsewhere. The true nature and the cause of the disease have baffled pathologists for 30 years. It was thought that an examination of the changes in plant composition connected with the disease might throw some light on its nature.

The symptoms of this blight have been adequately described by SHAPOVALOV (7). Briefly, vegetative growth ceases; the leaflets become leathery and the margins roll upward; the stems, petioles and leaf veins become purpled, and the upper surface of the leaf becomes more or less yellow. Affected plants are non-productive and usually die after a few weeks. Occasionally, however, regeneration of part or all of a blighted plant occurs in the field. Efforts by the writer to induce regeneration by cutting back the tops, and by spraying with nitrates and compounds of iron have given negative results. Stem cuttings of blighted plants placed in tap water and in nutrient solutions often produced roots but no vegetative growth.

For analytical purposes, samples were collected at Davis in June, 1925, of roots, stems and leaves from healthy and blighted plants of the Earliana variety. Other samples were collected in June and July, 1926, of the San Jose Canner variety. All samples were taken about 11 A. M., during clear hot weather. The samples of June 8, 1925, were taken at the beginning of an attack that killed 95 per cent. of the plants within the next few weeks; those of June 18, 1925, were of plants that had been definitely blighted for ten days or more. The samples of 1926 were taken at the beginning of two mild attacks which together destroyed only 5 per cent. of the plants. The sample designated as "incipient blight" showed slight external indications of the disease for several weeks. It eventually recovered, however, and bore a normal crop.

The samples of January, 1926, are of special interest. These were taken at Riverside in Mr. SHAPOVALOV's plots. The plants were those of the preceding summer's crop, which had escaped a moderate attack of blight,

* Contribution no. 17, Division of Truck Crops, University of California.

¹ Acknowledgment is made to F. S. HENIKA for performing the analytical work.

and due to absence of frost continued growth till midwinter. All of the plants in this field, except in 2 plots, assumed in early winter the yellowish-purple color and the non-vegetative condition characteristic of blight. One of the exceptional plots was fertilized with sulphate of ammonia, the other received seepage from a leaky sewer. The plants in these plots were dark green and vigorously vegetative. Because of the known deficiency of nitrogen in the soil, these latter have been termed "ample nitrogen" plants, the former "nitrogen starved" plants. Since the nitrogen starved plants had the external appearance of blight, it was of interest to determine if this condition could be differentiated chemically from true blight.

Plant samples were preserved in boiling 95 per cent. alcohol (with CaO). After drying the solid portion of the sample, and grinding, extraction was completed with 50 per cent. alcohol. Carbohydrates were determined by the SCHAFFER-HARTMAN method—sucrose after inversion with invertase (1 cc. in 100 for 2 hours), and starch after autoclaving for one hour and digestion with taka-diastrase over night. Total nitrogen was determined by the official salicylic-sulphuric acid method, observing the precautions advised by RANKER. Nitrogen was determined separately on aliquots of the insoluble and alcohol-soluble portions of each sample. The per cent. of the total nitrogen occurring in the soluble fraction is also given. The amino nitrogen in the alcohol extract was also determined but the results are not presented; in general amino nitrogen constituted about one fourth of the alcohol-soluble fraction.

Table I gives the changes in absolute amounts of certain constituents in healthy and blighted plants. It is evident that the Western Yellow Blight of tomato is characterized by very definite changes in composition. One of these is the increased percentage of dry matter in all parts of blighted plants. The changes in relative amounts of the different constituents (as shown when the results are calculated to the dry weight basis), are somewhat smaller than those given on the fresh weight basis. However, it is readily seen that there are marked changes in composition, both relative and absolute.

In the leaves, reducing sugars, sucrose, and starch increase progressively with the development of external symptoms of blight. It is probable that some of the striking symptoms of blight, *e.g.*, purpling of stems and veins, and rolling of the leaves, are connected with the high carbohydrate content. The purple pigment, anthocyanin, is well known to become intensified in the presence of high sugar concentration.

There is a very decided decrease of total nitrogen in blighted leaves; this decrease is entirely in the insoluble fraction. In fact, the amount of soluble nitrogen actually increases, and the per cent. in soluble form in-

TABLE I
COMPOSITION OF HEALTHY AND BLIGHTED TOMATO PLANTS—AS PERCENTAGE OF FRESH WEIGHT

DATE	MATERIAL	DRY MATTER	REDUCING SUGAR	SUCROSE	STARCH	NITROGEN			
						TOTAL	INSOL- UBLE	SOLUBLE	PER CENT. SOLUBLE
Leaves									
June 8, '25	Healthy	15.1	0.62	0.39	1.11	0.608	0.518	0.091	14.9
June 8, '25	Severe blight	18.6	1.91	0.73	3.52	0.440	0.320	0.120	27.3
June 18, '25	Very severe blight	18.9	3.19	1.36	2.18	0.457	0.302	0.155	33.9
June 18, '26	Healthy	15.0	0.38	0.20	2.25	0.675	0.576	0.099	14.6
June 18, '26	Incipient blight	16.1	0.65	0.09	3.61	0.595	0.481	0.114	20.0
June 18, '26	Severe blight	20.1	1.40	0.38	6.48	0.485	0.341	0.144	30.5
July 24, '26	Healthy	12.5	0.78	0.10	1.23	0.585	0.495	0.090	15.4
July 24, '26	Recently blighted	15.9	1.21	0.25	3.42	0.414	0.303	0.111	26.8
Jan. 8, '26	Ample nitrogen	12.7	0.18	0.29	2.85	0.557	0.441	0.116	20.9
Jan. 8, '26	Nitrogen starved	18.5	1.12	1.08	7.42	0.482	0.400	0.082	17.0
Stems									
June 8, '25	Healthy	9.6	0.94	0.31	0.74	0.201	0.119	0.083	41.3
June 8, '25	Severe blight	13.9	2.06	1.15	3.41	0.279	0.161	0.117	42.0
June 18, '25	Very severe blight	15.0	2.31	1.36	1.22	0.372	0.196	0.177	47.6
June 18, '26	Healthy	8.0	0.35	0.06	0.37	0.231	0.085	0.146	63.2
June 18, '26	Recently blighted	11.0	1.85	0.92	1.10	0.284	0.127	0.154	54.2
Roots									
June 8, '25	Healthy	15.3	0.35	0.74	0.82	0.280	0.183	0.096	34.4
June 8, '25	Severe blight	17.1	0.65	1.42	1.18	0.389	0.256	0.133	34.2
June 18, '25	Very severe blight	15.6	0.70	2.44	2.34	0.387	0.176	0.211	54.4

creases markedly. It seems as though upon the inception of blight, the movement of nitrogen into the leaves is stopped before growth is entirely halted, thus diluting the nitrogen already present and reducing the per cent. of nitrogen in these tissues. The accumulation of carbohydrates and other solids is not sufficient to account for the reduction in per cent. of total nitrogen. There seem to be other changes, also, which result in some of the more complex insoluble nitrogen constituents being converted back to soluble forms.

In the stems and roots of blighted plants, the accumulation of sugars and starch is almost as marked as in the leaves. Hence it is apparent that the disease involves all parts of the plant. It also seems that translocation of carbohydrates is not interfered with, but that their utilization in tissue-building is. The accumulation of carbohydrate is due mostly to the stopping of growth by some other unknown agency. While carbohydrate accumulation gives to the blight disease some of its characteristic symptoms, it is probably not the primary cause of the trouble.

In contrast to the leaves, total nitrogen in stems and roots shows an increase in the case of blight, this increase being especially noticeable in the roots. However, the proportion of soluble nitrogen increases in these tissues as it does in leaves, but not at the expense of the insoluble fraction. It appears, then, that blight is not due to nitrogen deficiency, for blighted plants continue, at least in the early stages of the disease, to take up nitrogen from the soil solution, though they are not able to translocate it into the leaves.

Attention may now be called to the two leaf samples taken in January at Riverside. The nitrogen starved plants, which externally had the appearance of Western Yellow Blight, likewise showed the extreme accumulation of sugars and starch characteristic of blight. As would be expected, total nitrogen is also somewhat decreased in the nitrogen-starved plants. However, the soluble nitrogen is also decreased somewhat, as is also the proportion of total nitrogen in soluble form, compared to that in the "ample nitrogen" or normal plants. Also the amino nitrogen is decreased from 3 per cent. of the total N. in normal to 1.9 per cent. in starved plants. Hence it seems that blighted tomato plants may be distinguished from those whose growth is checked by other causes, through their nitrogen relations.

It was thought that the starch accumulation in blighted plants might be connected with the inactivation of the natural plant diastases, hence determinations were made of the diastatic activity of healthy and blighted plants. Fifty grams of fresh ground tissue were extracted with 75 cc. glycerine (+ toluol) at 12° C. for 24 hours. This was made to 250 cc. and filtered. Aliquots of the filtrate were used to determine diastatic activity.

To one aliquot, 25 cc. of 1 per cent. soluble starch was added. Another aliquot, without starch, was run as a check, under similar conditions. After incubating 48 hours (24 hours in second experiment) at 30° C., the solutions were cleared and sugars determined. Gain in reducing sugar indicates diastatic activity in the plant material. The amounts are expressed as milligrams of dextrose, in table II.

TABLE II

DIASTATIC ACTIVITY OF LEAVES OF HEALTHY AND BLIGHTED PLANTS

FIRST EXPERIMENT, JUNE 18, 1926	EXTRACT + STARCH	EXTRACT ALONE	GAIN
	mg.	mg.	mg.
Leaves of healthy plants.....	491.0	153.5	337.5
Leaves of incipient blight.....	591.0	396.0	195.0
Leaves of severe blight.....	1000.0	898.0	102.0
SECOND EXPERIMENT, JULY 24, 1926			
Leaves of healthy plants.....	223.7	145.6	78.1
Leaves of blighted plants.....	506.8	483.7	23.1
Stems of healthy plants.....	278.7	270.6	8.1
Stems of blighted plants.....	603.1	603.1	0.

It appears that diastatic activity is greatly reduced in the leaves of blighted plants, but does not disappear entirely. In healthy stems diastatic activity is surprisingly low, and it seems to have disappeared in blighted stems.

Discussion

As previously stated, the cause of Western Yellow Blight is not known. SHAPOVALOV (7) has shown that the regional as well as the seasonal occurrence of the disease is associated to a very marked degree with weather conditions. Severe losses from blight occur under conditions conducive to high evaporating power of the air. SHAPOVALOV also records the favorable effect of shading upon the inhibition of blight, under conditions where otherwise it would have been very destructive. The writer has also observed cases where slight shading, as from young orchard trees, reduced the amount of blight. Since the evaporation rate would be little influenced by such transitory shading, some connection with the light factor is indicated. The disease generally appears in summer when the days are long, and in regions where the sunlight is particularly intense because of the cloudless days and clear atmosphere. The work of ARTHUR *et al* (1) and of Miss PFEIFFER (6) is also of interest in this connection. These workers found that tomatoes

grown with varying length of day evidenced injury with a daily light period of 17 hours or longer, indicating that the tomato is particularly sensitive to light. Miss PFEIFFER's long-day plants were dwarfed, yellowish, and were high in carbohydrate with decreasing protein. These changes resemble those found in blighted plants, though the magnitude of the changes is much greater in the case of blight.

On the other hand, McKAY and DYKSTRA (5) state that there is a causal relationship between the curly top disease of beets, and blight of tomato. It is true that the regional and seasonal occurrence of the two diseases show considerable parallelism. Furthermore, in the original publication of BALL (2) on curly top of beet are found statements indicating light-relationships for that disease similar to those which have been observed in tomato blight.

The chemical changes connected with curly top of beet were studied by BUNZEL (4). Though he reports many analyses, there are few strictly comparable data in his report, and BUNZEL made no attempt to draw conclusions therefrom. However, it seems that in general, in the leaves of curly top beets, there is a reduction in sugar content and no effect upon the nitrogen. In curly top roots there is a reduction in sugar, while total and insoluble nitrogen increases. Except for the last mentioned item, the changes in curly top beets seem to be the opposite of those found in blighted tomatoes. Of course this is not absolute evidence against the theory of a common causal agency in the two diseases.

BREWER, KENDRICK and GARDNER (3) have studied the changes in the tomato plant accompanying the two virus diseases, mosaic and streak. These workers analyzed composite samples of the whole plant. They found that mosaic and streak infection led to reduction in per cent. of dry matter and of carbohydrates, and unchanged total nitrogen. All these changes are different from those found in the case of Western Yellow Blight.

Conclusions

Definite changes in chemical composition characterize the disease of tomato called Western Yellow Blight. As all parts of the plant show these changes, it appears that the disease involves the whole plant.

Carbohydrates accumulate progressively in all parts of the blighted plants. It is suggested that this accumulation is the result of inability to utilize the carbohydrates, vegetative growth having been stopped by some unknown cause. The carbohydrate accumulation is no greater than it is in plants stunted through nitrogen starvation or "hardened" by exposure to low temperature, hence it cannot be considered the primary cause of growth cessation or of blight.

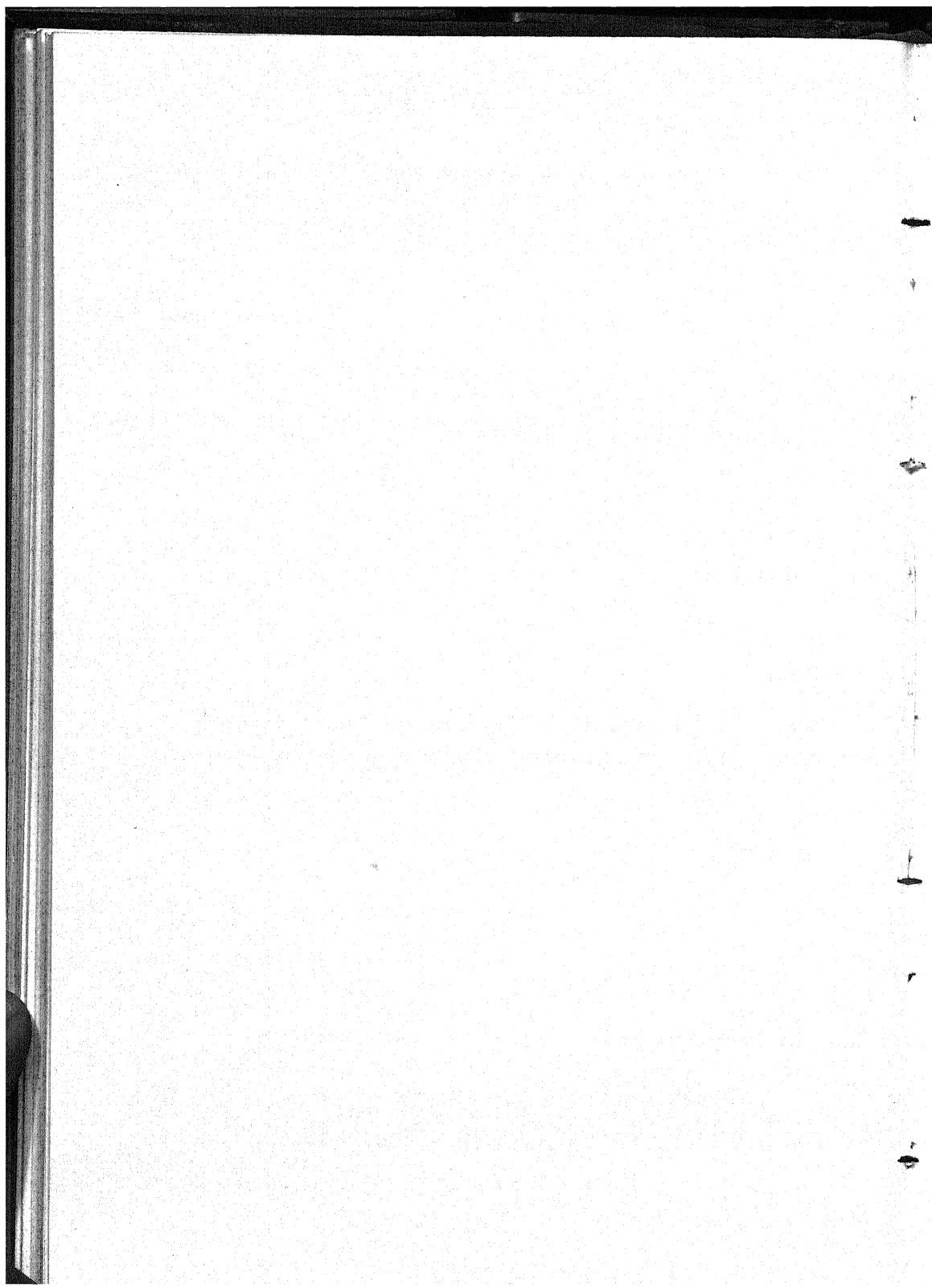
The total nitrogen content decreases in the leaves but increases in other parts of the blighted plant. Protein nitrogen was not determined as such,

but can be assumed to decrease, as the per cent. of nitrogen in soluble form increased in all parts of blighted plants. Nitrogen starvation can not be the cause of blight, but inability to translocate nitrogen within the plant, and inability to synthesize higher nitrogen compounds, may be more directly connected with the inception of blight.

UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA.

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THE EFFECT OF ULTRAVIOLET RADIATION UPON YEAST CULTURE MEDIA

J. W. WOODROW, A. C. BAILEY AND E. I. FULMER

(WITH ONE FIGURE)

While a great deal of work has been done on the effect of ultraviolet radiation upon the growth of yeast and other microorganisms, the effect of the rays upon the culture medium itself, so far as its growth-promoting properties are concerned, has not been adequately studied. It is obvious that the effect of the ultraviolet rays upon the organism in the medium may be due to a combination of factors, the direct effect upon the organism and an indirect action due to changes in the composition of the medium. This communication presents data on the effect of ultraviolet radiations upon the ability of several culture media to support the growth of yeast.

Equipment and methods

The source of the rays was a Cooper-Hewitt quartz lamp operated on a direct current of 110 volts and 4 amperes. Wratten filters were used in some cases to control the range of radiations used, and clear quartz "Vitreosil" Erlenmeyer flasks and Pyrex flasks were used as culture vessels.

The culture of *Saccharomyces cerevisiae* had been growing continuously for a period of years upon synthetic media. The counts were made with the Thoma-Zeiss chamber. When the count is one there are 250,000 cells per cubic centimeter. All incubations were made at 30° C.

The intensity of the ultraviolet was measured in lithopone units according to CLARK (16). This unit is defined as the energy required to darken the lithopone paste to a reflection factor of 50. Exposures of the media were made at 16 cm., under which condition six seconds were required to produce the standard darkening.

The media used were C, D and E as developed and described by FULMER, NELSON and SHERWOOD (18). The composition of these media, in terms of grams of solute per 100 cubic centimeters of medium, is shown in table I.

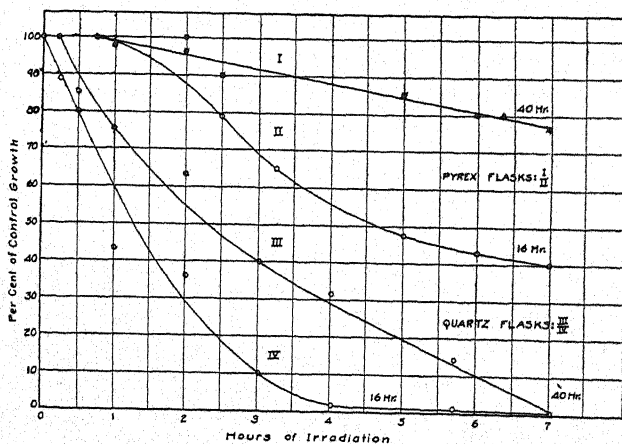
Each flask was weighed with contents before inoculation so that any evaporation during sterilization or irradiation could be compensated by the addition of the required amount of sterile water. In each instance 25 cc. of the medium were used in a 50 cc. quartz or Pyrex flask. The flasks were tipped at an angle so as to receive a maximum amount of the radiant energy.

TABLE I
COMPOSITION OF YEAST MEDIA USED

CONSTITUENTS	MEDIUM		
	C	D	E
	gm.	gm.	gm.
NH ₄ Cl	0.188	0.188	0.188
K ₂ HPO ₄	0.100	0.100	0.100
CaCl ₂	0.100	0.100
CaCO ₃	0.040
Sucrose	10.000	10.000	10.000

Experimental results

The results obtained for medium E irradiated for various time intervals are shown in figure 1. For comparative purposes the growth in the non-irradiated control flask has been taken as one hundred. These results show that with both the quartz and Pyrex flasks irradiation causes the development of toxicity in the medium, the effect being much greater for the quartz. These results are typical of many experiments. Irradiation of media C and D gave the same general results both for quartz and Pyrex flasks as those for medium E. Evidently the presence of the calcium chloride or carbonate does not influence results.



In order to determine whether heat rays had any part in the development of the toxicity in the medium these rays were filtered out by about 4 cm. of slowly running water. The receptacle containing the water was 6.25 x 12.5 cm. by 6.25 cm. deep. A quartz plate 2 mm. thick served as the

bottom of this filter, which allowed the ultraviolet radiations and the visible light rays to pass on through. Beneath this filter was placed a screen with an opening 5 x 5 cm. Across this opening were placed quartz, pyrex, and Wratten light filters, the results with the Wratten filters being tabulated in table II. These filters were ten centimeters from the lamp, and the flasks were ten centimeters from the filters. The energy reaching the flasks must be considerably less than it would be without the filter system. Nevertheless, even the short time irradiations, with the infra red rays filtered out, gave reduced counts equivalent to counts resulting from much longer irradiations when these rays were present.

In table II data are presented showing the growth of yeast in medium E exposed in quartz flasks for 30 minutes through Wratten filters under the conditions which have been outlined.

TABLE II
WRATTEN FILTERS, 30 MINUTES IRRADIATION

FILTER NO.	WAVE LENGTH AT MAXIMUM IN- TENSITY	RANGE OF FILTER	COUNT
	mμ	mμ	20 hours
17	350	300-425	7.6
34	420	315-500	5.8
47	440	350-530	16.0
40	500	450-630	21.6
Control	24.6

It is apparent that the toxic effect of irradiation is more severe with the shorter wave lengths, the effect being small for lengths longer than 440-500 mμ.

The data plotted in figure 1 show that after 40 hours of incubation the toxic effect in some cases is not so evident as after the 16-hour period. This may be due to the acclimatization of the yeast or to the disappearance of the toxic agent on standing. To test the latter point, flasks were irradiated for various lengths of time and either allowed to stand for various periods up to one week or were vigorously boiled. In neither case did the toxicity diminish to an appreciable extent. Evidently the toxic agent is non-volatile.

By irradiating separately distilled water, a solution of the salts, and the sugar solution, it was evident that the toxicity was entirely due to the effect of the ultra-violet rays upon the sugar. Similar toxicity developed by the irradiation of solutions of dextrose, calcium gluconate, or glycerol. Irradiated air with or without the removal of the ozone did not cause the development of toxicity nor was the toxicity a pH effect.

The effect of ultra-violet light upon the carbohydrates in solution has been studied by BERTHELOT and GAUDECHON (7, 9), BIERRY, HENRI and RANC (14, 15), BIERRY and HENRI (12, 11), and RANC (20). The following decomposition products are reported on long exposure (40-70 hrs.): H_2 , CO, CO_2 , CH_4 , HCHO. Sucrose is inverted and an acidity develops. BIERRY, HENRI and RANC (13) report that the exposure of glycerol to the ultra-violet leads to the formation of β -acrose in an alkaline medium. The reaction of other organic compounds has been studied by BERTHELOT (1), BERTHELOT and GAUDECHON (2, 3, 4, 5, 6, 8, 10), EULER and LINDBERG (17), and LOMBARD (19).

Although it has been shown here that the toxic agent is non-volatile it seemed advisable to study the action of CO on the medium. The gas was bubbled through the medium for various lengths of time. The one hour and three hour treatment cut the growth to 75 per cent. and 60 per cent. of the control, a toxicity not comparable with the irradiation. Moreover the toxicity disappeared on boiling. Evidently the toxic material is not carbon monoxide nor the result of the action of that substance upon the medium.

Summary

Upon exposure to ultra-violet light the yeast media developed a toxicity which increased with the length of time of irradiation. The toxic factor is non-volatile. The effect of ultra-violet upon yeast in these media must be due in part at least to alteration in the composition of the medium and not entirely to the direct effect upon the organism. In these experiments the toxic factor results from the action of the short rays on the sugar in the medium. For the study of the direct action upon the cell, a medium must be used whose growth-promoting properties are not changed by the ray treatment.

LABORATORIES OF PHYSICS AND BIOPHYSICAL CHEMISTRY,
IOWA STATE COLLEGE,
AMES, IOWA

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SEMI-PERMEABILITY OF SEED COVERINGS AND STIMULATION OF SEEDS*

FELIX KOTOWSKI

In a previous paper (6) the writer assumed that the methods of seed stimulation, advocated by POPOFF (9) are of little value for agriculture and horticulture [*cf.* also BREDEMAN (1)]. Many publications witness the great interest on the subject of seed stimulation. The occurrence of stimulation itself and the effect of so-called stimulants upon yield is still in controversy. However, the search for principles involved has been meager. These investigations aim to contribute to the knowledge of one of these principles, which is not the least important in the writer's opinion. The well established fact of semipermeability of seed coverings was overlooked by POPOFF and his associates. In this paper the term seed coverings is used to include all structures outside the embryo and endosperm. The significance of the semipermeability of the seed coats was stated by SHULL (11, p. 191): "The testa has physical and chemical characters, which may enable it to modify greatly any factors entering into germination behavior and the effects of these characters must be known before any sound conclusions can be drawn. Semipermeability is now shown to be common enough that its existence should be proved or disproved before proceeding to use stimuli acting through membranes." This was written ten years before POPOFF started to publish his papers on the topic of seed stimulation.

POPOFF (9) claimed to explain the real cause of stimulation by reagents. Unfortunately, he and his associates when presenting successful results did not give the exact composition of the stimulants used and therefore their work is not susceptible of verification, but it is certain that they have paid no attention to the permeability of the seed coverings.

Methods

Samples of seeds (50–80 gm.) were washed in tap water five minutes and rinsed two minutes in running distilled water. In this way, probably all salts on the surface of the seeds were removed. After washing, the seeds were dried 12 hours at 40° C. and used 12 hours later. Two and one half gram samples of seed were soaked 16 hours in 25 cc. of the solutions

* Work done at Davis, Calif., Division of Truck Crops, University of California, while on leave from College of Agriculture, Warsaw, Poland.

used, in 100 cc. flasks. The checks were similarly soaked in distilled water, after which the samples were divided into two groups.

In one group, the seeds were separated from the liquid, dried and weighed, and the gain of weight calculated as percentage of the air-dry weight. A uniform procedure was adopted for this drying. The seeds were blotted with filter paper for one minute and then later dried with a cloth for one minute. After weighing, the seeds were immediately soaked in 25 cc. of distilled water for 4 hours and were again dried and weighed. In the second group of samples, the seeds after being soaked 16 hours in solutions or in distilled water were removed from the liquid and rinsed in running distilled water for one minute; the further proceedings were the same as in the first group. The electrical conductivity of the solutions, and of the distilled water in which the seeds were immersed, was determined as soon as the soaking stopped.

The standard deviation of the weights was 2.5 per cent. of the mean, working with 4 replications of each kind of seed and for each solution.

The intake of water was almost complete after 16 hours; the gain during the next 4 hours was very small (0.03 cc. average), therefore the calculations have been made on the basis of the 16-hour water intake.

The intake of salts by the seeds was determined by the measurement of electrical conductivity of the external solutions. This method shows minute differences in concentration of aqueous salt solutions and detects small amounts of salts accurately and promptly. Water of high degree of purity was used ($C = 2.8 \times 10^{-6}$ mhos) and the measurements and computations were made according to FINDLAY (4).

Since the rate of water and salt intake and the processes of diffusion are influenced by temperature, the experiments were run at $20 \pm 1^\circ$ C. The conductivity measurements also were made at $20 \pm 0.1^\circ$ C. by means of KOHLRAUSCH's method. A dipping electrode was used in the solutions.

STILES (12, 13, 14) used conductivity determinations in the study of permeability of plant tissues, but so far as the writer is aware this method has not been used in permeability experiments with seeds. The results obtained by the conductivity method may need revision before conclusions can be drawn.

The corrections to be made were reported by STILES and KIDD (12) and in so far as they can refer to seeds, may be briefly stated. The possible causes making for a fall of conductivity in the external salt solutions aside from absorption, and which therefore make the values obtained greater than the true values for absorption, are as follows: 1. Reactions between the exudate and the external solutions by which non-ionized molecules are produced. 2. An increased exosmosis due to the action of the salts on the

tissues, as compared with this phenomenon, is distilled water. 3. An independent absorption of ions (*cf.* also PANTANELLI, 8).

These experiments were affected with these three sources of error, but estimation of any of them is impossible, although in the case of seeds, most important is the diffusion of carbon dioxide evolved by respiration. The check series (soaked in distilled water) aimed to estimate this error, but it was merely assumed that the respiration process was equal in distilled water and in solutions, which may not be entirely true.

The value for intake or for diffusion of salt was found from the equation: $C_2 = C_2 - (C_1 + C_x)$; in which C_2 is the final conductivity of the solution, C_1 the initial conductivity of the solution, C_x the increased conductivity caused by life processes of seeds, determined in check series; for distilled water $C_1 = 0$.

The diffusion of salts from seeds soaked and washed for one minute as well as from soaked and unwashed seeds was compared. The diffusion from washed seeds was lower, and the difference was considered as the loss due to washing for one minute. This loss represented the minimum quantity of salt that did not penetrate the seed coverings. The technique used for this purpose was very gentle indeed when compared to that of WOLFE (16), who says that LiCl (saturated solution) was only superficially absorbed by barley grains, however, since it could all be removed by an *hour's* washing in running water.

Two salts, the kations and anions of which are claimed by POPOFF to be good stimulants, were chosen. KNO_3 and $MnSO_4 \cdot 4H_2O$ in 1.50 per cent. aqueous solutions were used for soaking the seeds. Their conductivity at 20° C. was as follows: For 1.5 per cent. (= 0.15 N) KNO_3 , $C_1 = 33.80 \times 10^{-3}$ mhos; for 1.5 per cent. (= 0.067 N.) $MnSO_4 \cdot 4H_2O$, $C_1 = 13.20 \times 10^{-3}$ mhos.

Viable seeds of *Spinacia oleracea inermis*, *Cucumis sativus*, *Capsicum annum*, *Triticum vulgare*, *Secale cereale* and of *Hordeum distichum* were tested. The seeds were carefully selected to eliminate injured ones, but in cereals besides the selected (A) grains, non-selected (B) samples of commercial seed were tested. The examination of the latter showed 20 to 30 per cent. of grains more or less injured in barley and rye, and 5 per cent. in wheat.

Results

ABSORPTION AND PERMEABILITY FOR KNO_3

The absorption of KNO_3 and water by these seeds, and the loss of salt by exosmosis from seeds of the various plants used, are shown in table I.

The highest amount of salt was taken in by spinach and pepper (2.1 per cent. of the weight of seeds); lower was the gain for wheat A and B, rye B

TABLE I
 SAMPLES 2.5 GM. SOAKED IN 1.5 PER CENT. KNO_3 (=0.15 N) AT 20° C. FOR 16 HOURS

CHANGES OBSERVED	SPINACH	CUCUMBER	PEPPER	WHEAT A	WHEAT B	RYE A	RYE B	BARLEY A	BARLEY B
Salt absorbed, mg.	50.90	10.40	50.30	25.00	24.30	11.70	25.00	8.50	27.10
Water absorbed, cc.	2.30	1.22	2.34	1.02	1.23	1.40	1.46	1.00	1.08
Concentration of salt in seed, per cent.	2.21	0.85	2.16	2.45	1.98	0.84	1.71	0.87	2.49
Loss of absorbed salt due to exosmosis during 4 hours, seeds unwashed, as percentage of amount absorbed	60.00	76.50	80.00	54.50	35.00	54.00	33.00	78.00	29.00
Loss of absorbed salt due to exosmosis during 4 hours, seeds washed, as percentage of the amount absorbed	15.70	24.00	13.80	9.00	18.80	31.50	17.40	24.40	13.00
Loss of absorbed salt due to the washing of seeds one minute, as percentage of the amount absorbed	44.30	52.50	66.20	45.50	16.20	22.50	15.60	53.60	16.00

and barley B (1 per cent. of the weight of seeds); the intake of KNO_3 by cucumber, rye A and barley A is very moderate (0.4 per cent. of the weight of seeds). The intake of salt is in some degree dependent upon the intake of water. This fact is shown by the values for the final concentration of the salt within the seeds after 16 hours of soaking. On the basis of these figures we can classify the seeds as follows: First, barley B and wheat A, having final inside concentration of 2.47 per cent. compared with a final external concentration of 1.45 per cent; then pepper, spinach, wheat B and rye B, with an inside concentration of 2.01 per cent. against 1.45 per cent. outside concentration. All these seeds possessed higher concentration of KNO_3 within the seeds than outside, when the soaking was stopped. A reverse case could be noticed in cucumber, rye A and barley A; for these seeds the final inside concentration of salt was 0.85 per cent. against 1.53 per cent. of the external solution.

It is very obvious that the uninjured grains of barley and rye showed slight intake of KNO_3 in comparison with the injured ones. This was not the case with wheat, for which, however, SCHROEDER (10) found impermeability of seed coverings against KNO_3 .

The behavior of whole grains of barley and rye as well as those of cucumber leads to the conclusion that the seed coverings checked the intake of salt and its passage into the tissues of the seed within the layers of the seed coverings.

As far as wheat, pepper and spinach are concerned, it is rather difficult to explain their greater intake of KNO_3 as caused by permeability of seed coverings. It seems more justifiable to credit this to the layers of testa and pericarp in which the salt was accumulated. Here we meet a problem that is very important in connection with the methods of seed stimulation used by POPOFF, and an attempt was made to determine the localization of the salt taken in by the seeds.

To secure some reliable data, studies of the leaching effect of distilled water were made. The seeds were removed from the salt solution, dried, and soaked four hours in distilled water. This represented only one fourth of the time spent in the salt solution, but diffusion was great enough to be detected by conductivity measurements. The losses due to this process were calculated in percentage of the salt taken up by the seeds (*cf.* table I). They were different for seeds previously washed and for unwashed seeds. We will first consider the unwashed seeds.

The relation between the intake of salt and the ease of its removal from the seeds is plain. When the ions of KNO_3 penetrated the embryonic seed tissues, as was the case in samples B of the cereals, the exosmosis was small, and dropped to about half of that loss which was shown by the samples A

(32.3 versus 62.4 per cent.). The vegetable seeds, being all carefully selected, showed losses even greater than those of cereals A, *i.e.*, 72.2 per cent., which indicates that their seed coverings were chiefly responsible for absorption of KNO_3 , although the salt was accumulated in a higher degree than by cereals A.

This is corroborated by the behavior of washed seeds. The rinsing of seeds was exceedingly short (about 0.001 of the time for the intake of salt), nevertheless, it was possible to get distinct differences in diffusion, when washing took place.

After washing, during the same time and in the same conditions, diffusion was decreased for spinach, cucumber and pepper from 72.2 to 17.8 per cent. (loss due to washing 54.4 per cent. of the intake of salt); for cereals A from 62.4 to 19.3 per cent. (loss due to washing 43.1 per cent.); and for cereals B from 32.3 to 16.4 per cent. (loss due to washing being only 15.9 per cent. of the intake of salt). The low numbers for cereals B are in good agreement with our assumption that KNO_3 was really absorbed by grains with injured seed coverings and therefore elimination of the salt from the tissues was not possible by short washing. In the other groups in which intact seed coverings prevented the penetration of KNO_3 to the endosperm and embryo, the salt was easily removed, indicating the superficial character of its intake. Accordingly, there is little probability, if any, that in vegetable seeds and in cereals A the kation and anion could act on the protoplasm and stimulate growth (*cf.* also KOTOWSKI, 6).

ABSORPTION AND PERMEABILITY FOR MnSO_4

The absorption of MnSO_4 and water, and exosmotic loss of salts from the seeds used, are shown in table II.

The seeds of spinach and pepper showed the largest quantities of salt taken in (2.1 per cent. of the seed weight). The gain in salt by barley A and B and wheat B was one half of this (1.1 per cent. of seed weight) and the minimum amount was absorbed by rye A and B and cucumber (0.8 per cent. of seed weight). For barley A and B, the final inside concentration was 2.91 against 1.43 per cent. final outside concentration. A lower gradient was found in spinach, pepper, and wheat A and B (2.22 versus 1.44 per cent.). The external solution was stronger than the internal only for rye A and B and for cucumber (1.38 versus 1.5 per cent.). The seeds of vegetables were similar in their intake of MnSO_4 and KNO_3 .

In the case of cereals, the difference between samples A and B was small. The intact seed coverings checked but little the intake of MnSO_4 , which in quantity exceeded KNO_3 . Each cereal seemed to exhibit a specific behavior toward the intake of MnSO_4 , apparently with little regard to the seed coverings.

TABLE II
 SAMPLES 2.5 GM. SOAKED IN 1.5 PER CENT. $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ (= 0.007 N) AT 20° C., 16 HOURS

CHANGES OBSERVED	SPINACH	CUCUMBER	PEPPER	WHEAT A	WHEAT B	RYE A	RYE B	BARLEY A	BARLEY B
Salt absorbed, mg.	53.10	18.70	52.50	21.60	27.50	16.20	21.00	26.80	33.40
Water absorbed, cc.	2.30	1.22	2.34	1.02	1.23	1.40	1.46	1.00	1.08
Concentration of salt in seed, per cent.	2.30	1.54	2.24	2.12	2.24	1.16	1.44	2.72	3.10
Loss of absorbed salt, due to exosmosis during 4 hours, seeds non-washed, as percentage of the amount absorbed	39.00	34.60	57.50	43.50	18.70	37.00	35.00	21.00	21.70
Loss of absorbed salt, due to exosmosis during 4 hours, seeds washed, as percentage of the amount absorbed	9.00	12.60	19.20	12.90	7.50	15.20	15.00	13.20	9.60
Loss of absorbed salt, due to the washing of seeds one minute, as percentage of the amount absorbed	30.00	22.00	38.30	30.60	11.20	21.80	20.00	7.80	12.10

STILES and KIDD (14) reported that bivalent ions are at final equilibrium absorbed to a much less extent than the monovalent ions. In their experiments the tissues of carrot were soaked for 42, 64.5, 71.5 and 91 hours. It can be concluded in the present investigation that the 16-hour period of soaking was far from equilibrium, as to the salt, and one can apply rather the initial order of the rate of absorption, in which first place is always given to K. Anions appeared, according to STILES and KIDD (14), to be absorbed most rapidly at first in the order: SO_4 , NO_3 , Cl, but later in the order: NO_3 , Cl, SO_4 . This observation fits with our case, as far as the cereals are concerned, if we suppose that SO_4 was the ion more absorbed from MnSO_4 and K from KNO_3 . It was not possible to determine which properties govern the rate of intake of MnSO_4 in cereals, and it would be premature at present to make a suggestion.

Considering now the losses due to diffusion of salt from unwashed seed, we record for vegetable seeds 43.7 per cent., for cereals A 33.8 per cent., and for cereals B 25.1 per cent. loss.

The judgment upon the character of the intake of MnSO_4 was supported by examination of the diffusion from seeds washed for one minute. A decrease was found, however, smaller than for KNO_3 . The vegetable seed showed a diminution from 43.7 to 13.6 per cent., and the loss of salt due to washing was 30.1 per cent.; for cereals A, the decrease was from 33.8 to 13.8 per cent., the loss due to washing being 20 per cent.; for cereals B the diffusion changed from 25.1 to 10.7 per cent., the loss due to washing being 14.4 per cent. The percentages obtained in this case were all lower than those found for the diffusion process when KNO_3 was used. These results indicate that these two salts act in some ways differently on the seeds in question.

The ions of MnSO_4 entered into the seeds in larger quantities and moreover were not so easily withdrawn as the ions of KNO_3 . This may be seen from the following comparison. If the losses due to diffusion from unwashed vegetable seeds in case of $\text{MnSO}_4 = 1$, then we have for KNO_3 losses = 1.6; the washing process produces a loss of $\text{MnSO}_4 = 1$ as against 1.8 for KNO_3 . In the samples of cereals with intact seed coverings only, the ratios for unwashed seeds are 1 : 1.8, and for washed seeds are 1 : 2.1, respectively. But little difference occurred within the samples of cereal grains with some seed coverings injured. The amount of eliminated salt from unwashed seeds with injured seed coverings was for $\text{MnSO}_4 = 1$ and for $\text{KNO}_3 = 1.3$, and the losses due to washing were as 1 (MnSO_4) : 1.1 (KNO_3). In the last group it is clear that the washing process exercised the same effect on the seeds soaked in solutions of both of the salts. From this presentation of ratios we can say that the limiting factor for the penetration of KNO_3

and MnSO_4 was the impermeability of seed coverings. When this factor was not operative the salts reached the embryo protoplasm and there was a real absorption by tissues other than testa and pericarp.

The results of these experiments indicate the important rôle played by the seed coverings in questions of seed stimulation.

GOLA (5) was the first to demonstrate and announce the existence of a selective semipermeable layer in seed coverings, and in an examination of the seeds of 500 species, belonging to some 40 families, such membranes were found to be regularly present, except in the *Leguminosae* and in certain genera of the *Cistaceae* and *Cruciferae*. BROWN (2, 3) made the first quantitative study of the phenomenon, especially on barley seeds, and various other workers contributed extended quantitative studies made on other seeds; notably MAREL (7) on squash, SCHROEDER (10) on wheat, SHULL (11) on cocklebur; TJEBBES (15) claimed that seeds of the sugar beet probably have a selectively permeable membrane as a part of the inner seed coat.

The papers of BROWN, MAREL, and SCHROEDER deal with solutions of high concentration. But the permeability of seed coverings to the dilute solutions of salts applied as stimulants has never been proved to the writer's knowledge.

Summary

The effect of 16 hours' soaking in 1.50 per cent. solutions of KNO_3 and $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ was studied for 9 lots of seeds. The intake of the salts by the seeds, and the diffusion of the salts from the seeds in subsequent soaking, was determined by means of electrical conductivity measurements of the external solutions. From the data recorded, the following conclusions may be drawn:

1. The intake of salt depends on the kind of seed and on the cation and anion of the salt, but the limiting factor is apparently the seed coverings.
2. The seed coverings exercised a distinct check in the intake of KNO_3 , especially by cereals.
3. When the seed coverings were intact, absorbed salts were held superficially. Accordingly, heavy losses in the amount of salts absorbed resulted when the seeds were washed one minute.
4. The samples of cereals with grains partially injured showed high intake of salt and slight losses due to washing, owing to the permeability of spots deprived of seed coverings.
5. The behavior of seed coverings in the presence of chemical stimulants must be known in order to escape failures with seed stimulation in work done on larger scale.

The writer wishes to acknowledge the support afforded him through a research fellowship granted by the International Education Board to perform this investigation.

COLLEGE OF AGRICULTURE,
WARSAW, POLAND.

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THE USE OF ETHYLENE, PROPYLENE, AND SIMILAR COMPOUNDS IN BREAKING THE REST PERIOD OF TUBERS, BULBS, CUTTINGS, AND SEEDS

G. A. VACHA AND R. B. HARVEY
(WITH ONE PLATE)

It has been known for some time that ether (8, 4, 9), chloroform, ethyl bromide (1, 5), and other substances (6) may be used for breaking the rest period of certain plant materials. Since the time of undertaking the study here reported, the use of ethylene for a similar purpose has been advocated by ROSA (7), and DENNY (2, 3) has published upon the applications of ethylene chlorohydrin, thiourea, potassium thiocyanate, and other substances in breaking the dormancy of potatoes. DENNY did not find ethylene or propylene especially effective in breaking the dormancy of potatoes. In this study trials of the comparative activity of ethylene, propylene, and ethylene oxide in breaking the rest period have been made with a number of seeds, tubers, bulbs, and cuttings.

Breaking the dormancy of potatoes with ethylene

In making tests of potatoes for the presence of mosaic by the tuber index method it is desirable to secure as uniform germination of the samples as possible. It is desirable to know within as short a time as possible after digging, the degree of freedom of the seed samples from mosaic diseases so that they may be judged as to grade. The potato has a rest period during which it will not sprout even if placed under favorable conditions.

Selected tubers of Early Ohio, Rural New Yorker, Bliss Triumph, Burbank Russet, Green Mountain, and Irish Cobbler were obtained from A. G. TOLAAS, in charge of the Minnesota Seed Potato Certification. The samples of these varieties were kept uniform in growth in the field and in storage conditions preliminary to trial.

The tubers were divided into four lots, and six tubers of each variety were selected for each lot, or, in other words, each lot contained six varieties of potatoes and six tubers of each variety. The tubers were carefully selected so as to have the lots as uniform in size as possible. Only those tubers free from mechanical injury and disease were used.

Lots 1 and 2 were then put into culture ovens where the temperature was maintained at 20° C. A beaker full of H₂O was set inside each oven to keep the air moist.

Lot 1 was then treated with ethylene gas, one part to one thousand by volume, while lot 2 was used as a check. At the end of thirty-six hours lot 1 received the second dose of ethylene. At the end of seventy-two hours the third dose was applied, and a fourth dose after 120 hours. The total length of treatment was six days.

A cylinder of ethylene oxide was cooled to 0° C. to liquefy it, and an aqueous solution of 1 cc. of ethylene oxide to 1,000 cc. of water was prepared. Lot 3 was soaked in this solution for twenty-four hours. Lot 4 was soaked for twenty-four hours in water. Both lots were held at room temperature.

At the end of the treatment the tubers were spread out and dried quickly by blowing a stream of warm air over the tubers. After drying, each lot was cut by means of a cork borer into pieces three-fourths of an inch in diameter. This gave a very uniform sized seed piece having an average weight of one-fourth ounce. The seed pieces were allowed to dry for two hours before planting.

Thirty seed pieces were then selected from each variety and planted in a cutting bench in the greenhouse. The seed pieces were planted in rows eight inches apart, and four inches apart within the row. Depth of planting was two inches. The soil used for planting was of the 3-2-1 mixture, light clay, sand, and leaf mold. All four lots received bottom heat, the same amount of light, and about the same quantity of water. Data were first recorded when the sprouts appeared above the surface of the ground, and at other intervals. Table I indicates the marked difference in the length of the dormancy among the various varieties as shown by the number sprouting at fifteen and forty-eight days. Evidently, the Early Ohio variety has the longest period of dormancy, followed by Rural New Yorker, Green

TABLE I

EFFECT OF ETHYLENE ON SPROUTING OF POTATO VARIETIES

VARIETY	NUMBER APPEARING ABOVE GROUND AFTER PLANTING ¹			
	15 days		48 days	
	Ethylene Lot 1 ²	Check Lot 2 ³	Ethylene Lot 1 ²	Check Lot 2 ³
Early Ohio	0	0	27	23
Rural New Yorker	2	0	28	25
Bliss Triumph	18	3	30	29
Burbank Russet	19	4	30	30
Green Mountain	4	1	29	29
Irish Cobbler	8	4	30	30

¹ 30 seed pieces of each variety were planted.

² Treated with ethylene 1:1,000.

³ Check kept in air six days.

Mountain, and Irish Cobbler. The majority of the seed pieces which received the ethylene treatment had sprouted in twenty days, except the Early Ohio. The majority of these appeared above ground in twenty-eight days after planting. In the case of lot 2 (check) a longer time was required to obtain complete sprouting, namely thirty-five days. This would indicate that the ethylene treatment speeded sprouting from seven to fifteen days.

In lot 4 the tubers soaked in H_2O for twenty-four hours required thirty-five days for complete sprouting. Hence soaking the tubers in water had no effect on dormancy. Of the tubers in lot 3 soaked in a water solution of ethylene oxide (1:1,000), only few sprouted, which indicates that ethylene oxide is very toxic. Most of the seed pieces were decayed when dug up.

In each case the ethylene-treated seed pieces, when once above the ground, grew much faster than the untreated ones. This is very clearly demonstrated by the photographs, figures 1, 2, 3, 4, 5, Plate I. The great stimulation in all of the tubers treated with ethylene is noticeable. Only one set treated with ethylene oxide is shown (table II), because most of

TABLE II

EFFECT OF ETHYLENE OXIDE ON SPROUTING OF POTATO SEED PIECES

VARIETY	NUMBER APPEARING ABOVE GROUND AFTER PLANTING ¹			
	15 days		48 days	
	Ethylene oxide Lot 3 ²	Check Lot 4 ³	Ethylene oxide Lot 3 ²	Check Lot 4 ³
Early Ohio	0	0	0	25
Rural New Yorker	1	1	1	26
Bliss Triumph	2	2	2	30
Burbank Russet	3	1	4	30
Green Mountain	0	1	2	26
Irish Cobbler	0	4	0	28

¹ 30 seed pieces of each variety were planted.

² Treated with ethylene oxide 1:1,000 in water for 24 hours.

³ Check immersed in water 24 hours.

the seed pieces rotted. Evidently this ethylene treatment will offer considerable advantage in obtaining rapid growth for the determination of tuber index for the mosaic diseases.

Treatment of corms and bulbs to break the rest period

The growing of gladiolus in greenhouses during the winter months is not a profitable business on account of the long time required for germina-

tion. For this reason only a limited amount of gladiolus is grown under glass. Commercial florists have expressed a desire for growing more gladiolus under glass if a way to treat them to shorten the length of the rest period could be found.

Forty corms of gladiolus were selected and arranged into five lots; twenty corms to lot 1 and five corms to each of the other four. The corms were carefully selected so as to be free from disease and injury, and they were as uniform in size and weight as possible. All the corms were put into air-tight glass-stoppered bottles for treatment as follows:

Lot 1. Kept in air.

Lot 2. 2 cc. of ethyl ether were added to 100 cu. inches air space.

Lot 3. 2 cc. of chloroform were added to 100 cu. inches air space.

Lot 4. Received ethylene gas, one part to one thousand parts of air.

Lot 5. Received propylene gas, one part to one thousand parts of air.

The six jars were put into a culture oven where the temperature was maintained at 20° C. After six days the corms were removed from their respective jars and planted in flats of soil.

TABLE III

EFFECT OF TREATMENT WITH ANAESTHETICS UPON SPROUTING OF GLADIOLUS

LOT	TREATMENT	NO. OF CORMS TREATED	NO. GROWING 32 DAYS AFTER PLANTING
1	Check	20	1
2	Ether	5	5
3	Chloroform	5	4
4	Ethylene	5	4
5	Propylene	5	3

As shown in table III, most of the treated corms were growing, while only one of the checks was above ground thirty-two days after planting. This experiment was repeated with a larger quantity of corms. Practically the same results were obtained. On account of the difficulty of getting larger numbers of corms of the same variety which were exactly of the same maturity, only the trial with perfectly uniform corms is reported. These experiments indicate that the dormant period of gladiolus corms was reduced by one half. It is estimated that the growth was advanced twenty-five to thirty days.

It is interesting to note that at the concentrations used ether seems to be most efficient in breaking dormancy, followed by chloroform. No differ-

ence was noted between the ethylene- and propylene-treated corms. Evidently these two homologues are about equally effective. However, the corms which received the ethylene and propylene treatment seem to grow faster than those treated with ether and chloroform, probably on account of some injury by these latter substances.

In later experiments on the larger lots the average height was determined, giving for ether- and chloroform-treated $5\frac{1}{2}$ inches while the plants treated with ethylene and propylene averaged $8\frac{1}{4}$ inches tall. Ethylene oxide, concentration 1:1,000 was also used, but it appears to be very toxic and kills the tissues.

Dahlia tubers and cuttings of cannas were treated with ethylene 1:1,000. Ethylene did not seem to hasten growth in the dahlias, but it did stimulate the development of a greater number of buds on each root of cannas. On each cutting of canna which received a dose of ethylene two to four buds developed, giving two to four plants of good vigor, while the checks with few exceptions gave only one plant per cutting.

Effect of ethylene and propylene on hardwood cuttings

Hardwood cuttings of apple, plum, cherry, golden willow, red osier, lilac, alpine currant, grape, cottonwood, common elder, high bush cranberry, mock orange, honeysuckle, and pear were treated with ethylene and with propylene (1:1,000). Two doses were given, the second dose following forty-eight hours after the first application.

The dormancy of all of the above-mentioned twigs was broken so that leaves developed, and in the case of apple and plum flower buds also developed. There seems to be very little if any difference between ethylene and propylene in their ability to break the dormancy of buds.

Effect of ethylene and propylene on the germination of seeds

Dormant seeds of common buckthorn, high bush cranberry, snowberry, and Tartarian honeysuckle were treated with ethylene, 1:2,000, 1:1,000, 1:500, and 1:200 in air. Also propylene was used in the same concentrations. Buckthorn seeds were soaked for two minutes in concentrated H_2SO_4 , washed thoroughly in H_2O , and finally washed in a two per cent. solution of sodium carbonate. These seeds were then treated for six days with ethylene 1:500. They gave almost a perfect germination in thirty-five days. Buckthorn seeds receiving a treatment of ethylene 1:500 alone showed fifty per cent. germination of the seeds in thirty-five days; slightly less than fifty per cent. germination when a concentration of 1:1,000 was used. Ethylene or propylene 1:500 for eight days in three doses gave good germination in the case of dormant seeds of high bush cranberry,

snowberry, and Tartarian honeysuckle. The use of concentration higher than 1:500 produces some injurious effects.

Conclusions

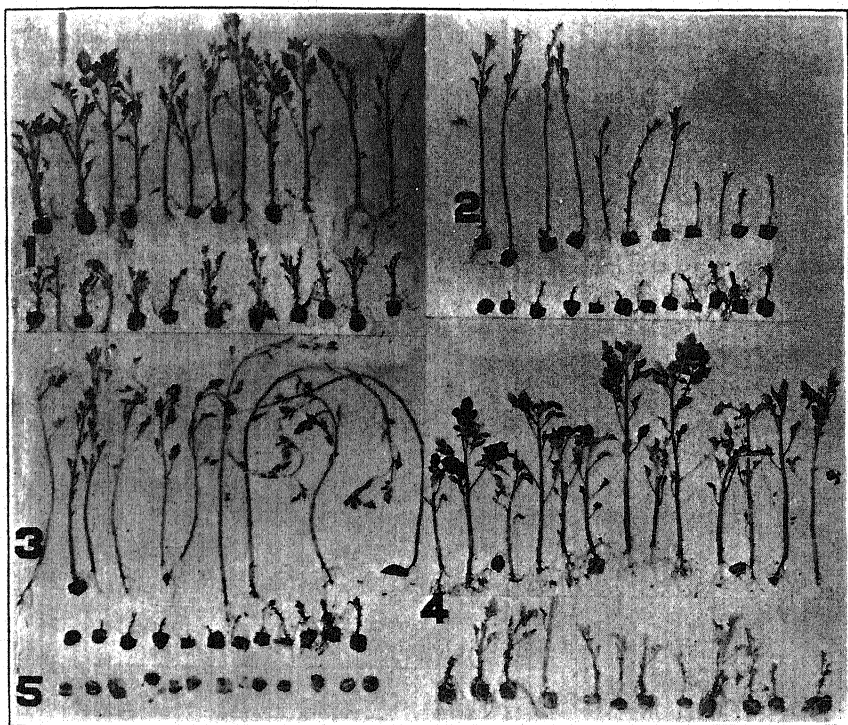
The dormancy of potato tubers varies in length, being longest in the case of Early Ohio and shortest in Bliss Triumph and Burbank Russet.

Ethylene at a concentration of one part of the gas to one thousand parts of air by volume breaks the rest period and hastens the sprouting of tubers. The time gained by such a treatment varies from seven days in the case of the Early Ohio variety to eight or nine days for Green Mountains and Rurals, to fifteen days for Burbank Russet and Bliss Triumph. The treated tubers grow faster than untreated ones. The growth stimulation by ethylene and by propylene is shown to be greater than the stimulation of ether and chloroform in gladiolus. Ethylene oxide, one part in one thousand parts of water, was toxic to potato tubers and to gladiolus. Ethylene, either alone or after treatments by sulphuric acid, is effective in securing germination of seeds of buckthorn, high bush cranberry, Tartarian honeysuckle, and snowberry. Ethylene and propylene were found about equally effective in breaking dormancy.

MINNESOTA AGRICULTURAL EXPERIMENT STATION,
ST. PAUL, MINNESOTA

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STIMULATION OF GROWTH OF POTATOES BY ETHYLENE

- FIG. 1. Early Ohio. Upper row, ethylene treated; lower row, check.
 FIG. 2. Green Mountain. Upper row, ethylene treated; lower row, check.
 FIG. 3. Burbank Russet. Upper row, ethylene treated; lower row, check.
 FIG. 4. Bliss Triumph. Upper row, ethylene treated; lower row, check.
 FIG. 5. Bliss Triumph. Ethylene oxide.

THE DETERMINATION OF SOLUBLE CARBOHYDRATES*

It is generally agreed that soluble carbohydrates are the most available, as well as the original, source of metabolic energy for plants. Hence, studies of the formation, translocation, condensation, hydrolysis and assimilation of these compounds have commanded, and will undoubtedly continue to command, a major place in plant physiological analysis. Separation between soluble and insoluble carbohydrates is here made by classing as insoluble those compounds which require hydrolysis before they can be quantitatively extracted from plant tissues by water or alcohol. In the strictest sense the extraction of dextrans and pentosans is probably accomplished by suspension rather than by true solution, but for analytical purposes they may be termed "soluble" compounds. In the interest of completeness we have included the glucosides under this heading, although it is hardly probable that they should be classed with the carbohydrates from the standpoint of physiological function.

A brief treatment of the occurrence, solubility, reducing power and other properties of the carbohydrates here considered is given by HAAS and HILL (4). All but pectins and the gums are readily soluble in water. These and the polysaccharides (dextrin, inulin and mannan) are insoluble in hot 80 per cent. alcohol. It is well known that all naturally occurring free sugars with the exception of sucrose and raffinose reduce Fehling's solution. The saponins also have this property. Other glucosides, the soluble polysaccharides, pectins and gums acquire reducing power by hydrolysis, as do the non-reducing sugars. Furthermore, such reducing disaccharides as maltose and lactose acquire an increase of this property by hydrolysis. The latter carbohydrate is added to tissue in the use of commercial taka-diastase, which may contain up to 75 per cent. of the sugar.

The presence of more than two or three per cent. of pentose sugars, or of appreciable quantities of galactose or mannose, is generally considered to be an indication of poor technique in preservation or separation, since these sugars are transition compounds normally present in polymerized forms. DAVIS and DAISH (2) are authority for the statement that the presence of maltose or isomaltose is due to starch hydrolysis in preservation and they object on this ground to preservation by drying. Apparently germinating seedlings may be an exception to this rule, but it is general enough in its application to be of value.

* Section III of the report of the Committee on Methods of Chemical Analysis of the American Society of Plant Physiologists.

Initial separations

Obviously, the initial separation of soluble carbohydrates depends upon a method of killing the tissue which will inhibit enzyme action. A method of extraction should then be used which will remove only those substances which can be determined together. When a limited number of substances are to be estimated it may be possible to make the initial separation with water. Generally, however, the first separation is best made with alcohol. Because of the rapidly increasing solubility of dextrin and other colloids at alcohol concentrations below 70 per cent., and the limited solubility of sugars in strong alcohol, an 80 per cent. solution of alcohol is commonly used. This extraction has the further advantage of generally separating colloidal (protein) and non-colloidal compounds of nitrogen, as well as colloidal and non-colloidal carbohydrates. The end point of the alcoholic extraction should always be determined. As a general rule, soluble carbohydrates will be removed as soon as, or sooner than, soluble nitrogen compounds and one test may thus be made for the two fractions.

A second extraction with water or 10 per cent. alcohol will remove dextrans, inulin and soluble pectins, together with a small percentage of hemicelluloses. In the absence of free fructose, acid hydrolysis may be applied to this fraction, whereas its use would be impossible if the first and second fractions were combined. This order of extraction is sometimes modified by using 50 per cent. alcohol to ensure complete extraction and then making the extract to 80 per cent. of alcohol to precipitate dextrans and other colloids.

Carbohydrates soluble in eighty per cent. alcohol

The alcohol fraction contains the substances classed as sugars and also the glucosides. If the material has been killed by dropping it into sufficient boiling 95 per cent. alcohol to give a final concentration of 75 to 80 per cent., and extraction completed at the same strength, the colloidal carbohydrates, pectins, etc., will be practically excluded. The presence of fructose, which is claimed by HAAS and HILL (4) to constitute more than half of the total sugars in many plants, governs the handling of this extract, and precludes the use of basic lead acetate in clearing, extended heating in acid hydrolysis, or any heating in the presence of a base or a lead salt.

CLEARING.—Although 80 per cent. alcohol precipitates many interfering colloids it extracts lipoidal substances, chlorophyll, tannins, and flavones which may make filtration almost impossible when the sample is taken up in water. In addition amino-acids and tannins reduce Fehling's solution. Tannins whenever present should be removed by clearing. Unfortunately no good method is available for removing amino-acids and where these substances are present in quantities an error is introduced into the reducing

and total sugar values. This error should be small with most types of plant materials.

Three materials have been in common use as clearing reagents. Alumina cream may be used to flocculate colloids, but is of no value in removing non-colloidal impurities. Neutral lead acetate is a convenient clearing reagent which removes tannic acid, pectins and many flavones, and flocculates colloidal materials generally. The work of LOOMIS (6) indicates that a considerable excess of neutral lead acetate may safely be used in the presence of fructose, and also that this material answers all clearing requirements in the removal of organic impurities, when the sugars are to be estimated by copper reduction. Basic lead was shown, in the same paper, to precipitate as much as 15 per cent. of the reducing substances present, without any apparent gain in clearing effectiveness. Potassium oxalate is recommended for deleading on the basis of the work of SAWYER (11), MEAD and HARRIS (8), and LOOMIS (7). Hydrogen sulfide and mono-sodium phosphate remove the lead more completely than potassium oxalate, but leave the solution acid. Sodium carbonate and disodium phosphate are effective deleading agents but their alkalinity is destructive to fructose, while the use of sodium sulfate is liable to entail difficulties in filtration.

Convenience in clearing usually requires that the lead precipitate be filtered off before deleading. Otherwise the flocculating effect of the clearing reagent is lost and filtration proceeds with difficulty. Reducing impurities may also be returned to solution. As stated above, fructose must not stand with lead acetate and the solution should not be raised above room temperature, either during clearing or after deleading, unless the last traces of lead are first removed.

REDUCTION.—Two methods of copper reduction are in general use, the two minute boiling of MUNSON and WALKER (9), and the longer heating at 80° C. of QUISUMBING and THOMAS (10). Any reduction procedure must be carefully standardized and the method of QUISUMBING and THOMAS appears to have the advantage in that the temperature does not vary with altitude and barometric pressure, and variations of a few seconds in heating time constitute a much smaller percentage error with the longer heating period. It is also claimed that auto-reduction and reduction by sucrose are avoided. The MUNSON-WALKER method has the advantage of complete tables but requires more attention. However, if a simple water manometer is attached to the gas line so that the gas pressure can be adjusted accurately, the heating conditions of the MUNSON-WALKER method can be maintained without great difficulty.

A common, six-hole water bath may be used in the QUISUMBING and THOMAS method if it is provided with a motor-driven stirring device and protected from air currents. Both the bath and the burners should be sur-

rounded by an asbestos board screen. With some experience the temperature may be manually controlled with considerable accuracy by means of adjustable pinch cocks and a gas pressure manometer.

In addition to the directions on preparation of crucibles given on pp. 190-191 of the revised methods of the A. O. A. C. (1) it is advisable to make one or more blank runs with new crucibles until their weight becomes nearly constant. The loss of important samples may be avoided in this way.

DETERMINATION OF REDUCED COPPER.—Direct weighing of cuprous oxide is generally the most convenient method of determining reduced copper for the inexperienced chemist. This method is not "official" for plant extracts but has been shown (6) to give comparable results when sufficient copper can be weighed to reduce the percentage error of the fluctuations in the weight of crucibles. As a general rule, reductions giving less than 20 mg. of copper should not be weighed directly, and even at this figure, the percentage error is high. With a sufficiently large cuprous-oxide precipitate, standard methods of calculation may be used with reasonable assurance of comparable, if somewhat high, results, or an empirical copper factor may be introduced to correct for the low percentage of copper in the precipitate from plant extract reductions. This percentage has been found to be roughly constant for a given lot of material and to vary around a value of 87.2 per cent. copper instead of the theoretical value of 88.8 per cent. (6).

Many laboratories employ the volumetric-permanganate method of determining copper and feel that with solutions standardized and some practice in titration, it is a more rapid method than direct weighing. With the proper precautions, particularly in standardization of solutions, potassium-permanganate titration should be preferable for small quantities of cuprous oxide because it eliminates the principal source of error in direct weighing, namely, the fluctuations in the weight of crucibles. The direct titration method of SHAFFER and HARTMAN (12) has given irregular results with plant materials. Apparently variations in character of the tissue may affect the end-point of the titration. Under such conditions, of course, the method is worthless. The committee feels that the SHAFFER and HARTMAN method should not be recommended for general use on plant material without further trial.

The picric-acid method of estimating reducing substances has been advocated for rapid work and for the estimation of minute quantities of sugars. As modified by WILLAMAN and DAVISON (13), only 1 ml. of dilute sugar solution is required for each determination. This is of especial advantage when limited quantities of tissue are available for analysis.

HYDROLYSIS OF DI- AND TRISACCHARIDES.—Sucrose is the disaccharide for which this step is usually intended and the increase in reduction following inversion is frequently reported as sucrose. Maltose, raffinose, melibiose

and various glucosides, if present in the solution, will also show increased reducing power after hydrolysis with acid. Proper killing methods can usually be depended upon to eliminate maltose from consideration. This is fortunate, since maltose cannot be hydrolyzed in the presence of fructose without destroying the latter sugar. Seedlings of starchy seeds are an exception to this rule and may contain several per cent. of maltose, but they apparently contain little free fructose. Raffinose and melibiose are of limited occurrence, but may be estimated by the official method for determining sucrose and raffinose in beet products [p. 187, revised methods A. O. A. C. (1)]. The presence of glucosides may introduce an appreciable error in the total sugar determination unless invertase is used to invert sucrose. Acid hydrolysis under HERZFELD's conditions [pp. 186-187, revised methods A. O. A. C. (1)], either 10 min. at 70° or 24 hours at 20° C., would seem to be applicable to alcohol preserved samples in the absence of glucosides. Unfortunately, glucosides are very widely distributed, and the justification of the use of acid on a particular material must rest upon the investigator. Commercial preparations of invertase are now available and detailed directions for their use are given in the revised methods of the A. O. A. C. (pp. 183-6).

GLUCOSIDES.—The glucosides as a class have received very little attention, although the work of HARVEY (5) on apple twigs indicates that in this instance, at least, the proportions of a glucoside seem to follow the total metabolism curve very closely. If glucosides accumulate as a result of metabolic activities, their increase should be a better measure of such activities than the disappearance of sugars, because it would be less directly affected by photosynthesis and hydrolysis.

The term glucosides includes a large number of compounds, some of which are little related beyond the fact that they are all combinations of a reducing sugar and some aromatic substance. They may react as weak bases (solanin only), or as weak acids, or they may be neutral compounds. In general they are soluble in water or dilute alcohol but not in absolute alcohol and are precipitated by basic lead acetate; the acid glucosides are also precipitated by neutral lead acetate, and all are hydrolyzed to reducing sugars and aromatic compounds by mineral acids or appropriate enzymes. The method of estimating a glucoside must, therefore, be specific as to both the glucoside to be estimated and the material from which it is isolated.

The method devised by HARVEY (5) for the estimation of phloridzin in apple wood is based upon the reducing value of the glucose liberated upon the hydrolysis of this compound. A portion of the solution in which sucrose has been inverted by invertase should be used. The difference in reducing value before and after hydrolyzing with 2 per cent. hydrochloric acid for fifteen minutes is calculated as glucose, multiplied by the factor 2.42 and

expressed as phloridzin. A similar method could probably be used for arbutin in pear wood. In the case of the saponins, which reduce Fehling's solution both before and after hydrolysis, some means of separation is required which is adapted to the particular compound being determined. The glucosides are of special interest, first because they may be erroneously reported as non-reducing sugars, and second because they offer a suggestive field for future physiological work.

Carbohydrates soluble in water but not in strong alcohol

The compounds in this group are present in the plant largely as colloids which are easily suspended in water, but are precipitated by the 80 per cent. alcohol used for extracting sugars. Some of them are removed quantitatively and may be estimated from this fraction. Others are only partially soluble and should be considered here only as impurities in the soluble, or as losses from the insoluble, fraction.

DEXTRIN.—Dextrin is generally present in small quantities in starch-bearing tissue. At certain stages, dextrin may form an important part of the weight of starch storage organs. DAVIS and SAWYER (3) report as much as 4 or 5 per cent. of dextrin, on a dry weight basis, from leaves of the Irish potato, collected just after midday. Ten per cent. alcohol is frequently used for separating dextrin from starch in the residue from the alcoholic extract. When 50 per cent. alcohol is used for the first extraction, the dextrin is recovered from the extract by making it to 80 per cent. and allowing to stand. Dextrin is not easily precipitated by basic lead acetate and this material is recommended for clearing the water extract when dextrin and inulin are to be determined separately from the same sample. Neutral lead acetate is preferable at other times. The cleared extract is delead, hydrolyzed for 2.5 hours at the temperature of boiling water with 2 per cent. hydrochloric acid, neutralized, and its dextrose equivalent determined by copper reduction.

INULIN.—This is a condensation product of fructose which is found in many Compositae and monocots sometimes associated with starch and dextrin, although usually occurring alone. Inulin is readily suspended in water, but not in dilute alcohol. An extract containing inulin should be cleared with neutral lead acetate unless it is desired to remove this compound in order to make a separate determination of dextrin. Inulin is hydrolyzed with weak hydrochloric acid and determined as fructose. Because of the nature of the product of hydrolysis, very careful heating is required. The Bureau of Standards workers employ 1 per cent. acid and heat at 70° C. for 35 minutes.

PECTIC BODIES.—Because of their partial solubility, pectic bodies may cause difficulty in water or dilute alcohol extractions. Some of these sub-

stances precipitate as a gel upon cooling and so are not truly soluble. For this reason their determination will be considered in another section; at the same time they may seriously interfere with the handling of the water extract, particularly from fruits, fleshy stems and roots, and may even make a water or weak alcohol extraction impossible. Difficulties may be minimized by extracting with water or 10 per cent. alcohol at room temperature and by clearing this extract with neutral lead acetate before hydrolysis.

GUMS, HEMICELLULOSES, ETC.—Many materials of a gummy nature are partially soluble in water and must be removed from the dextrin-inulin fraction. Neutral lead acetate is suggested for this purpose. This solubility may also affect the total acid hydrolyzable fraction. If, however, the water extract is filtered after cooling, the precipitate can be included in the determination of insoluble carbohydrates according to the outline given in another section of this report.

This report was organized by W. E. LOOMIS for the Committee.

C. O. APPLEMAN,
W. E. LOOMIS,
T. G. PHILLIPS,
W. E. TOTTINGHAM (chairman),
J. J. WILLAMAN.

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THE DETERMINATION OF NITROGEN IN RELATIVELY SIMPLE COMPOUNDS*

It is assumed that the forms of nitrogen to be determined are present in the aqueous extract of plant material, which has been freed from protein, or in the solution obtained by extracting with 80 per cent. alcohol. The total amount of nitrogen in such extracts is usually rather small, and it is present in several different compounds. In no case has a complete determination of the distribution of nitrogen been made. One of the most thorough studies of this kind is that of Vickery and his co-workers on alfalfa (10). Because of the uncertainty as to the individual compounds present, and the practical impossibility of determining them in routine analysis, it has become customary to estimate the amounts of nitrogen present in certain forms of combination.

The determinations made most frequently are: Total nitrogen, including nitrates if these are present; ammonia nitrogen; amide nitrogen; amino-acid nitrogen; and nitrate nitrogen. Less frequently, basic nitrogen and the nitrogen of proteoses are determined. Often the sum of the various forms determined is subtracted from the total nitrogen and the difference called "Rest," "Residual" or "Other" nitrogen. The amount of this fraction and its relation to plant behavior indicate the need of its further separation and study, but methods are not as yet available for this purpose. How many of the possible determinations are to be made in any given case will depend on the amount of material available and the purpose of the study. No methods can be described which are equally useful for all plant materials. In each case preliminary studies must be made to learn the applicability of the methods and the size of aliquots best suited for use in the various determinations.

In most of the determinations the nitrogen is converted into ammonia and estimated titrimetrically. The amounts to be determined are usually small. It is necessary therefore to use especial care in selecting reagents free from nitrogen, in avoiding contamination and in carrying through suitable blanks. Solutions of H_2SO_4 and of NaOH of 0.02N strength will be found convenient. Each cc. of such solutions is equivalent to 0.28 mg. of nitrogen. The alkali used should be free from carbonates, and both solutions should be made with CO_2 free water, and preserved in well seasoned bottles. They are conveniently connected by siphons to burettes

* Section IV of the report of the Committee on Methods of Chemical Analysis of the American Society of Plant Physiologists.

with three-way stop cocks, and the NaOH solution should be protected by a soda lime guard tube. The strength of the solution should be checked at frequent intervals, as even in well seasoned bottles, the alkali is likely to increase slowly in strength.

Total nitrogen

If nitrates may be present in the material a test should be made for them. The test should be carried out carefully and approximately quantitatively, so that in case only a trace is present a fair estimate can be made of its amount. A portion of the aqueous extract (if an alcoholic extract is used the alcohol is removed by evaporation and the residue taken up with water) is treated with a slight excess of saturated neutral lead acetate solution. The resulting mixture is then made just alkaline to litmus with NaOH. The heavy precipitate is filtered off. With most materials this treatment removes so much of the organic matter that the diphenylamine test for nitrate as described by WITHERS and RAY (11) can be used satisfactorily. Should the test be negative, it is advisable to repeat it on portions of the cleared solution to which enough nitrate has been added to equal one part per 100,000 and one part per 1,000,000. The test should detect one part per 1,000,000 of nitrate nitrogen in the presence of some organic matter. In pure solution it is much more sensitive than this.

A. IN THE ABSENCE OF NITRATES.—Any of the official modifications of the Kjeldahl method may be used (1). The determination of small amounts of nitrogen, 2 to 10 mg., and the consequent use of 0.02N solutions of acid and base, make rather extreme care necessary. The distillation may be made through block tin condensers, preferably using distilling bulbs and tubes of hard glass. It is desirable to have a very efficient trap of the Davison or similar type in the distilling bulb. Methyl red is very satisfactory as an indicator, and the end point is sharpened materially if the distillate is boiled and cooled before it is titrated.

B. IN THE PRESENCE OF NITRATES.—The nitrogen of nitrates is fixed by combination with an aromatic compound, such as salicylic acid. The nitro groups are reduced and the total nitrogen is then liberated as ammonia by the usual Kjeldahl digestion. This method gave very erratic results until it was found that it is satisfactory on dry samples but very unreliable if water is present.

RANKER (6) has made a study of this determination, and the method described below is taken largely from his work.

Reagents needed.—Concentrated H_2SO_4 , nitrogen free, 30 cc.
of which contain 1 gm. of salicylic acid.
Sodium thiosulfate crystals.
Concentrated NaOH, 50 per cent. by volume.

The Determination.—A suitable aliquot of the extract is pipetted into a Kjeldahl digestion flask. It is brought to exact neutrality and evaporated just to dryness on a steam bath. Thirty cc. of the sulfuric-salicylic acid mixture are added and mixed thoroughly. The flask is stoppered and allowed to stand at least an hour, preferably over night. Five gm. sodium thiosulfate crystals are added and the mixture is heated for five minutes over a low flame. After cooling, 7–10 gm. of K_2SO_4 or anhydrous Na_2SO_4 , and a pinch of $CuSO_4$ are added, and digestion and distillation are carried out as in the usual Kjeldahl procedure except that no Na_2S is necessary, and the digest is neutralized by adding 100 cc. of the 50 per cent. $NaOH$.

Amino nitrogen

A portion of the extract is evaporated on the water bath to remove alcohol. When the volume has become small, about 25 cc. of water may be added and the evaporation continued. This is repeated until all traces of alcohol have been removed. The material is transferred with water to a volumetric flask and diluted to volume. Such a volume of the original extract should be taken for evaporation, that, of the final volume, 4 cc. contains at least 1 mg. of amino nitrogen.

Amino nitrogen is determined by means of the VAN SLIKE (9) amino apparatus. Descriptions of the apparatus and the technique of its use, and the tables necessary for calculating the results are to be found in the standard texts of Physiological Chemistry, such as those of HAWK and BERGEIM and of MATHEWS. The micro size of apparatus can be used, with a 2 or 4 cc. sample. It may be desirable occasionally to use the larger reaction chamber with the micro-burette, so that a larger sample, up to 10 cc., may be taken.

If the solution is nearly colorless, and no great amount of buffer is present, amino nitrogen may be determined by the SØRENSEN formol titration method as described by JODIDI (4). Neutral formalin is added to the neutralized solution. Acidity develops due to the destruction of the base forming properties of the amino groups by the formaldehyde. The amount of nitrogen originally present in such groups is measured by the amount of base required to bring the acid solution to neutrality again.

Ammonia nitrogen

It is rarely the case that ammonia nitrogen is present in plant extracts in amounts sufficient to make a satisfactory estimation possible. The determination must be made, however, to serve as a blank in the determination of amide nitrogen. The ammonia is aerated or distilled from a slightly alkaline solution into a measured excess of standard acid and its amount determined by titration. Any volatile bases other than ammonia that may

be present are included by this method. The amount of such bases is usually very small and their inclusion is not objectionable if the result is to be used only as a blank for the amide determination. If a more exact estimate of ammonia as such is desired it may be determined colorimetrically in the distillate by the use of NESSLER'S reagent. So far as results have been reported, however, this method has not proven entirely satisfactory with plant materials. Further studies of the use of this method are needed. For rendering the solution slightly alkaline before distillation, a slight excess of magnesia cream may be used. At the boiling temperature even this mild reagent may liberate some amide nitrogen or other nitrogen easily split off, and thus make the results for ammonia too high. The distillation is better carried out at 40° to 50° C. in vacuum. Distillation in vacuum with alcohol and $\text{Ca}(\text{OH})_2$ is an excellent method described by VAN SLYKE (8) for removing ammonia from protein hydrolysates.

Amide nitrogen

This is freed as ammonia by boiling an aliquot of the solution under a reflux for 2.5 hours with 6 per cent. HCl. The acid is neutralized and the ammonia distilled and determined as described in the previous paragraph.

The amount of amide nitrogen is found by subtracting from the total amount obtained, the ammonia nitrogen from an aliquot of the same size.

The following semi-micro methods may be used on portions of the extract freed from alcohol for the amino nitrogen determination, provided it has been concentrated sufficiently so that 10 cc. contain at least 1 mg. of amide nitrogen.

Ammonia nitrogen.—Ten cc. of the concentrated extract are pipetted into a pyrex test tube 30 mm. x 200 mm. The tube is connected in the VAN SLYKE-CULLEN urea apparatus with a similar receiving tube containing a measured volume, 10–25 cc., of 0.02N H_2SO_4 . Six or seven drops of capryl alcohol (technical) are added to each tube to prevent foaming, and 15 cc. 52 per cent. K_2CO_3 to the sample. VAN SLYKE recommended aeration for 30 min. at such a rate that a total of at least 120 liters of air are used. As it is sometimes inconvenient to measure the rate of aeration it is safer to aerate at a rapid rate for at least 45 minutes. The excess acid may be titrated in the test tube, using the aeration tube as a stirring rod.

Amide nitrogen.—Another 10 cc. portion of the concentrated extract is pipetted into a pyrex test tube 30 x 200 mm. Two or three pieces of granulated pumice the size of a grain of wheat or smaller and 0.6 cc. conc. H_2SO_4 are added. The tube is heated to gentle boiling under a reflux condenser for 2.5 hours. This may be done easily by the low flame of a Bunsen burner if the tube rests on an asbestos board with a hole in it about one-

half the size of the tube. The solution is cooled and nearly neutralized with 40 per cent. NaOH, about 2.2 cc. being required. The ammonia is aerated as described under ammonia nitrogen except that 20 cc. of 52 per cent. K_2CO_3 are used. From the nitrogen found, that estimated as ammonia nitrogen is subtracted. The remainder is amide nitrogen. In both these aerations a trap tube containing H_2SO_4 (0.5 N is sufficient) should be inserted in order to remove ammonia from the air.

Nitrate nitrogen

The colorimetric determination of nitrate nitrogen in plant extracts presents unusual difficulties. It is necessary (1) to remove chlorides; (2) to remove organic compounds as completely as possible by clearing; (3) to destroy such organic compounds as cannot be removed by clearing. A method using the phenol-disulphonic acid reagent has been proposed by BURRELL and PHILLIPS (2). This method is admittedly cumbersome, and is troublesome, too, because of the extreme care which must be used in obtaining reagents free from nitrogen. Properly carried out it is capable of yielding excellent results. It is to be hoped that further study may make this method somewhat more convenient.

Nitrate nitrogen may be reduced to ammonia nitrogen by the use of DEVARDA'S alloy in alkaline solution. STROWD (7) has based a method for determining nitrates in plants on this fact. Two equal aliquots of the solution, made equally alkaline are distilled, one with, the other without the addition of DEVARDA'S alloy. The ammonia obtained in the former distillate in excess of that in the latter is taken to represent the nitrogen originally present as nitrate and nitrite. CHIBNALL (3) has used a similar method. BURRELL and PHILLIPS (2) have found a loss of nitrate nitrogen by this method in the presence of ammonia and amide nitrogen.

This loss may be avoided by determining nitrate nitrogen in the solution remaining in the test tube of the VAN SLYKE-CULLEN apparatus after the removal of amide nitrogen. The contents of the tube are washed into a Kjeldahl flask to a volume of about 125 cc. and boiled down to a small volume, 15-25 cc., but not to dryness. The residue is cooled, diluted to 300 cc., and a few drops of paraffine oil are added to prevent foaming. Finally 1 gm. DEVARDA'S alloy is added and the mixture is distilled at once through a Kjeldahl apparatus into an excess of standard acid. The K_2CO_3 present provides alkalinity suitable for the action of the alloy, the reduction is complete, and the ammonia recovered is a measure of the nitrate and nitrite nitrogen originally present. In case this method is to be used the amide hydrolysis must be carried out with H_2SO_4 , as some nitrate nitrogen is lost on boiling with HCl. At the beginning of the distillation the mixture

should be watched carefully, as the K_2CO_3 solution tends to foam badly just as it starts to boil in spite of the addition of paraffine oil. It may be necessary to remove the flame for a short time at this point. Once the boiling has well started, excessive foaming stops. This method has been used successfully in several cases, but doubtless its general applicability should be studied more thoroughly.

Humin nitrogen

During the acid hydrolysis for amide nitrogen humin nitrogen may be precipitated. Its amount may be determined by filtering the residue from the distillation of amide nitrogen with magnesia or lime, washing and estimating total nitrogen in the insoluble residue by the Kjeldahl method.

Basic nitrogen

The addition of phosphotungstic acid to the acidified filtrate from the humin determination results in the precipitation of basic nitrogen. The HAUSMANN method, as modified by OSBORNE and HARRIS (5) for the determination of basic nitrogen in protein hydrolysates may be adapted for this estimation.

Proteose nitrogen

CHIBNALL (3) precipitates proteoses from protein-free plant extracts by saturating the acidified solution with zinc sulphate. The precipitate is washed, redissolved, reprecipitated, washed again, redissolved and made up to a definite volume. Total nitrogen is determined in an aliquot of this solution by the Kjeldahl method.

This report was organized by T. G. PHILLIPS for the Committee.

C. O. APPLEMAN,
W. E. LOOMIS,
T. G. PHILLIPS,
W. E. TOTTINGHAM (chairman),
J. J. WILLAMAN.

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BRIEF PAPERS

AN APPARATUS FOR CONTROLLING THE FLOW OF NUTRIENT SOLUTIONS IN PLANT CULTURES

(WITH ONE FIGURE)

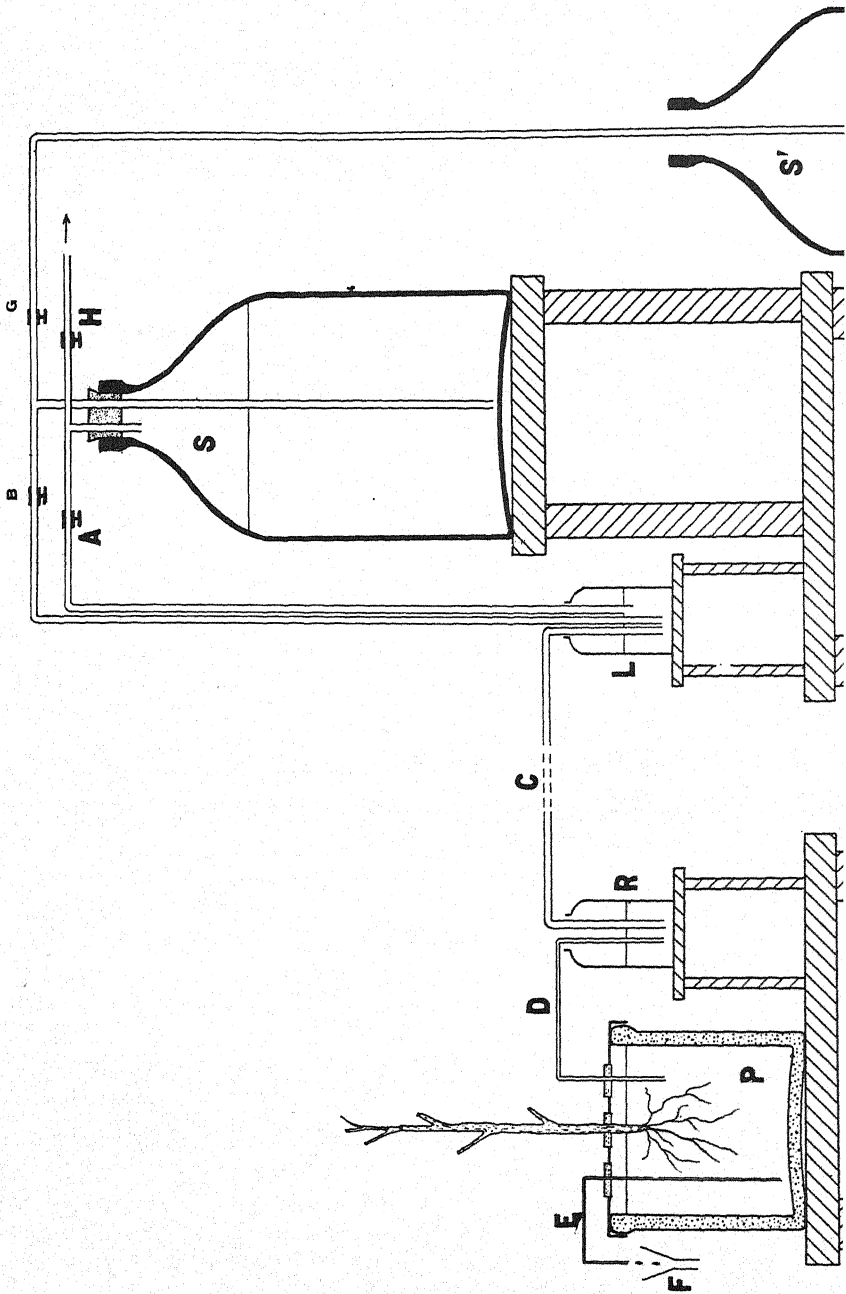
Each advance made in plant nutritional studies brings with it the realization that ultimately some satisfactory method of experimentation is yet to be devised whereby low concentrations of a given salt solution may be maintained in contact with the roots of plants. A solution of this problem may be approached by continuously renewing the culture medium. Several investigators¹ have already applied this method and described their apparatus. One phase of an investigation dealing with low potassium requirements of the tomato plant led to the devising of an apparatus of large capacity and flexible enough to be adapted to various types of experiments involving flowing solutions. All essential parts can be made and put together from materials usually found in plant physiological and chemical laboratories.

The two essential units of the apparatus are represented in the accompanying figure on tables *T* and *T'*. The plant container, *P*, is a 5-gallon glazed, earthenware jar. A special cover made of heavy sheet copper fits each of the 24 jars used in these experiments. Five holes were cut in each cover and then a heavy coating of tin was applied as a precaution against copper poisoning. These holes were large enough to receive the flat cork stoppers commonly used to support plants in ordinary fruit jars. The rate of flow from the plant container into the waste tube, *F*, is controlled by raising or lowering the siphon, *E*, which fits tightly into one of the cork stoppers of the cover. A delicate adjustment of stop-cocks is thus entirely eliminated. The rate of flow in one experiment was adjusted to approximately 7 liters per day per each jar, which in this case contained a single plant. Higher and lower rates can easily be had by simply adjusting the height of the outlet siphon.

¹ TRELEASE, S. F., and LIVINGSTON, B. E. Continuous renewal of nutrient solution for plants in water culture. *Science*, n. s. 55: 483-486. 1922.

ALLISON, R. V., and SHIVE, J. W. Studies on the relation of aeration and continuous renewal of nutrient solution to the growth of soybeans in artificial culture. *Amer. Jour. Bot.* 10: 554-566. 1923.

ANDERSON, F. G. A device for maintaining constant level of culture solutions. *PLANT PHYSIOLOGY* 1: 417-418. 1926.



In the experiment just noted, six 5-gallon jars were connected to a sub-reservoir, *R*, by means of siphons, *D*. As illustrated in the figure, this reservoir is connected by siphon, *C*, to the other unit of the apparatus, the constant level jar, *L*. A desired level is maintained by a modification of the well-known Mariotte flask. When a solution in *L* drops below the end of tube *A*, air enters the 10-gallon bottle, *S*, and with its partial vacuum thus destroyed fresh solution flows into *L* through the siphon *B* until the solution in *L* again rises high enough to enter tube *A* and attain a height that balances the partial vacuum in *S*. It is of course necessary that an air tight stopper be used in bottle *S* and that the stop-cocks, *G* and *H* be closed. Tube *A* should be approximately 7 mm. inside diameter with the lower end cut or ground at an angle. This prevents water from "hanging" in the tube when air should be entering. New solution is made up in bottle *S'* and then drawn up into bottle *S* by applying suction at *H* after opening the cocks *G* and *H* and closing *A* and *B*. It is well to paint the glass tubing and bottles black. This excludes enough light to prevent the growth of algae.

Attention is called to one essential difference between the types of apparatus heretofore described and the present one. In the former cases the rate of flow of nutrient solution is regulated before the solution enters the plant container. This is accomplished either by delicately adjusting stop-cocks or by passing the solution with a given hydrostatic head through proper lengths of capillary tubing. In the apparatus here described the outlet siphon controls the rate at which the solution flows from the plant container. This rate, together with that of transpiration, determines the rate of flow into this container. While the rate of flow through the outlet siphon remains practically constant for a given adjustment, that of transpiration may vary from hour to hour as well as from day to day. There is thus a definite amount of solution flowing from the plant container per unit of time which is always in excess of the transpiration by an amount depending on the adjustment of the outlet siphon.

It is a pleasure to acknowledge indebtedness to Professor D. R. HOAGLAND, of the Division of Plant Nutrition, University of California, for the facilities and equipment used in conducting the experiments involving the apparatus herein described.—EARL S. JOHNSTON, *Laboratory of Plant Physiology, University of Maryland.*

Fig. 1. Apparatus for controlling flow of nutrient solutions. Description in text.

DEVICES FOR SOWING AND GROWING SPORES

(WITH TWO FIGURES)

The usual but somewhat antiquated methods for sowing spores and small seeds in laboratory cultures results in great unevenness in distribution. In many places on the culture media the spores fall in masses and are then too dense for even growth, while other areas may be missed entirely. Despite much good work that has been done by these old methods, especially with certain spores, experience has shown that more uniform sowing of spores and seeds conduces to more normal growth and behavior of the structures that the spores produce. Prothallia and protonemata are capable of growing very densely and often do so in artificial cultures here referred to. Their ability to grow very densely exceeds that of most of the higher plants. However, such compact growths as are often seen when grown artificially are rarely, if ever, seen in nature owing to proper dissemination. Although it is clear that excessively dense growth in this respect is disadvantageous, a critical comparative study as regards the degree of influence exerted by growth density has yet to be made.

The writer, however, has constructed two devices which make it possible to sow spores very uniformly and to grow them in a better way than is possible by the usual methods.

The first of these, which is for sowing the spores, is shown in figure 1. It consists of a clear glass cylinder A, 5 cm. in length and has a uniform diameter of 16 mm. This size has been found convenient, although larger or smaller sizes of these glass cylinders are sometimes advisable, according to the volume of material available. A much smaller cylinder is preferable where only a small quantity of spores can be obtained. A cork B, one cm. in length and that just fits the cylinder closes the latter when not in use. Through the center of the cork is passed a glass tube C, having a bore of 4 mm., a length of 11 cm. and flared at its upper end. A cylinder of Swiss silk gritgauze D, 9 cm. long and that fits both cylinder and cork closely, is held to each of these by means of tight rubber bands, E. Such a cylinder of new gritgauze is stiff enough, if properly made, to support the weight of the glass cylinder when in a horizontal position and will not collapse easily. It is therefore more suitable than any other material, except metal gauze which, of course, should not be used for this purpose. The flared tube C is used to introduce spores into the sterilized glass cylinder without opening the latter. The tube is then plugged with cotton. It also assists

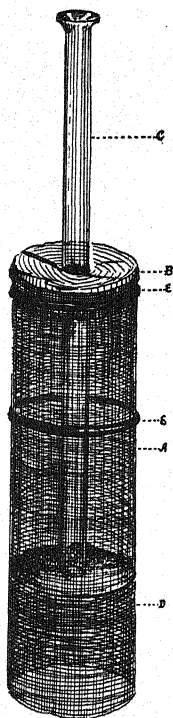


FIG. 1. Device for sowing spores.

in revolving the contrivance while sowing the spores and enables one to adjust the position of the glass spore container in the gritgauze cylinder D. A gritgauze cylinder having 4 to 6 meshes to the mm. sows fern or moss spores thickly enough if properly manipulated. For certain small seeds gritgauze having meshes 2 mm. square may be obtained. Figure 1 shows the apparatus closed, when it serves as a sterilized container. By shoving the glass tube C through the cork B, the desired adjustment of the glass spore container in the gritgauze cylinder is attained. In this way narrow or wide swaths of spores may be sown. When the apparatus is extended the spores are allowed to slide to the cork. The cylinder is then rotated in a horizontal position which allows the spores to be sown evenly and to the desired density. This should first be tested by passing the rotating cylinder over white paper. By means of this test it can be seen that great uniformity as to distribution is obtained. By holding the apparatus close to the sterilized culture medium while operating, the drifting of the spores by currents of air is avoided which is hardly possible by former methods. It is further advisable to place the culture inside of a sterilized glass case and then sow the spores.

The second device is for growing the spores as is shown in figure 2. It consists of a circular zinc box F, 25 cm. in diameter and 6 cm. deep. In the roof of this box is a circular opening G, the rim of which, as well as the periphery of the box, is turned up at right angles to a height of 12 mm. in order to hold a layer of distilled water for moisture control. The sides of the box F, are covered for part of their height by a strip of brass wire

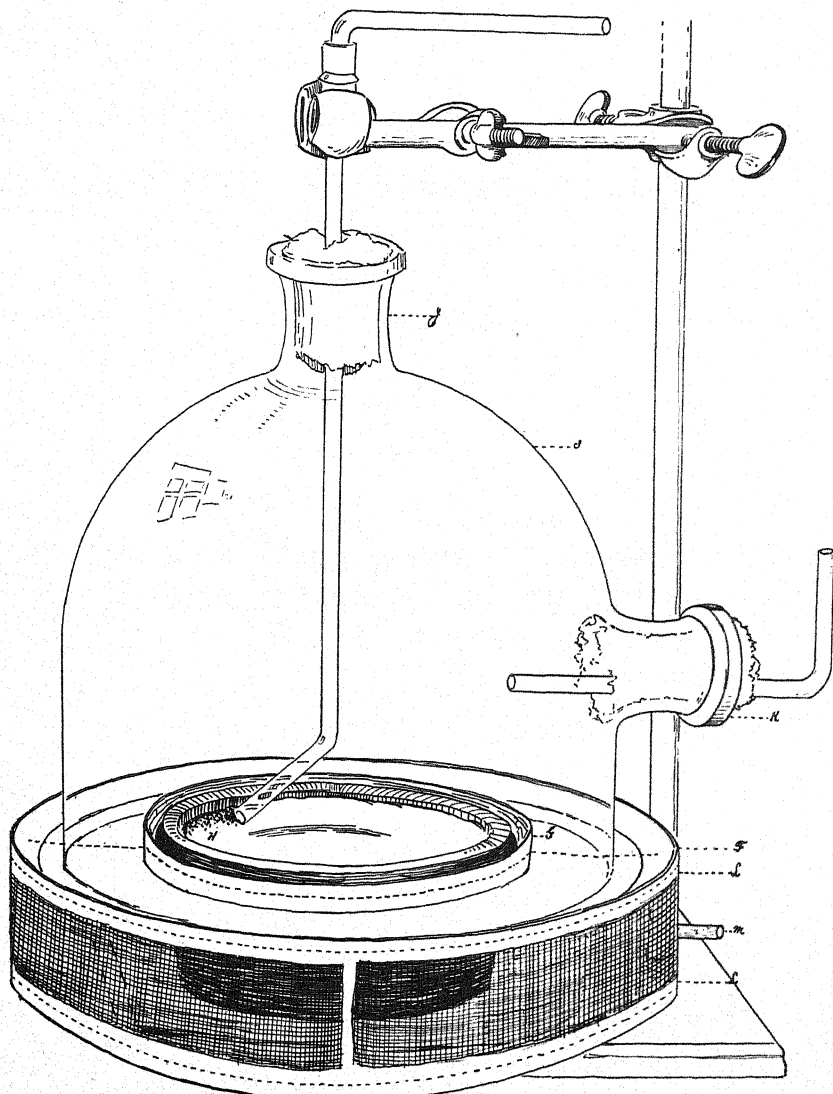


FIG. 2. Device for growing spores.

gauze having 8 meshes to the mm. and in addition this may be supplemented by a sterilized cloth covering, where necessary. The culture of spores H, sown as above indicated, is placed flush with the top of the central opening G. Over this is placed a low bell-jar I, with its lower edge submerged in the water. The shorter the bell-jar the better, but any tall bell-glass may be quickly cut off to the desired height by means of a number 20 nichrome wire carrying a 110 or 220 volt electric current which may be regulated by a rheostat. A terminal tubulure J, or better a lateral one K, carries a glass tube held in place by cotton or a rubber stopper according to requirements. This tube may be used for watering the culture without opening the apparatus, or for connecting with an aerating contrivance for the passage of air. If temperature control is necessary, a thermometer may be enclosed in I, and any desired temperature maintained. When carefully put together this piece of apparatus excludes the fungus-gnat and other insects, and obviates the danger of contamination, especially by moulds which often partly or completely destroy such cultures when grown by less controlled methods.

The fine brass wire gauze on the sides of F may be closed for certain experiments by a special contrivance. This consists of an adjustable metal draw-band L, wide enough to cover the brass wire gauze and provided with a tube M. It is coated with paraffin and made gas tight by means of plastilina or a suitable gasket on which the band is drawn tight. The interior of F should also be heavily coated with paraffin of a high melting point in order to insulate the surface of the metal, and a layer of paraffin oil substituted for the distilled water at G. For certain studies the bell-jar I may be cemented gas tight to the roof of F by a stiff air pump wax-mixture. The arrangement just described, especially when the spores are grown on a suitable substratum, extends the usefulness of the apparatus by enabling the operator to perform a great variety of experiments of physical or chemical nature directly on the spores themselves, or on the structures they produce during germination or during normal growth.—
F. M. ANDREWS, *Indiana University.*

IONIZATION AND ADSORPTION ISOELECTRIC POINTS

In considering the isoelectric points of membranes it is evident that these points may be produced in two manners. First, there is the commonly known isoelectric point produced by the ionization of ampholytes. Second, the membrane without going into solution appreciably may show an isoelectric point produced by differential adsorption. This fact of adsorption producing an isoelectric point has been largely overlooked in the discussion of the isoelectric point of membranes. F. E. BARTELL in his discussion on "Anomalous Osmose" in the First Colloid Symposium Monograph makes no definite statement of this although his graphs show that it exists. On calling this point to his attention I received a reply which I believe will be of interest to physiologists, which I quote here.

"In connection with solid material as particles or as membranes which appear to exhibit an electrical potential against water, I have assumed that an iso-electrical point is possible. This idea, I believe, has been carried along since the time of PERRIN's work. For example, in W. W. TAYLOR's "The Chemistry of Colloids," p. 69, referring to PERRIN's work, he states: "Negative diaphragms become more negative in alkaline solutions; in acid solutions the diaphragms become less negative, and with increasing concentration of acid they become electrically neutral and finally positive."

"I have regarded this electrically neutral point as a definite iso-electric point. In my past work I have accepted the HELMHOLTZ idea of the electrical double layer and have assumed that this layer (e) in aqueous solutions is largely the result of adsorption. By making use of this assumption many of the phenomena obtained with membranes appear to be fairly simple. As you are aware, FREUNDLICH has recently pointed out that the electrical double layer conception of HELMHOLTZ is not sufficient, that we must consider an electro-kinetic potential (ζ), which is not the same as the other. In case this latter view is correct, we can make use of the electro-kinetic potential and carry out the general treatment as before. Considerable work is now being done along this line by different investigators and undoubtedly we will have some worthwhile contributions appearing in the near future."

Will it make for better clearness to refer to these conditions as *ionization isoelectric points* and *adsorption isoelectric points*?—R. B. HARVEY, University of Minnesota.

NOTES

First International Congress of Soil Science.—In 1924 the Fourth International Conference of Soil Science held at Rome, resulted in the organization of an International Association of Soil Science. The first international congress of this Association will be held in Washington, D. C., under the presidency of Dr. J. G. LIPMAN, of Rutgers University, June 13–22, 1927. Four of the six International Commissions making up the Association deal with problems of vital interest to plant physiologists. These are the Commissions on Soil Physics, Soil Chemistry, Soil Bacteriology, and Soil Fertility. Arrangements have been made to present to the readers of *PLANT PHYSIOLOGY* an account of such features of the Congress as may be of interest to physiologists.

Fifth National Colloid Symposium.—The preliminary program of the fifth National Colloid Symposium, to be held at Ann Arbor, June 22–24, 1927, shows a very attractive program. Several of the papers should prove very valuable to students of plant physiology. Dr. GORTNER and his co-workers will discuss the proteins and the lyotropic series, MICHAELIS reports his investigations on molecular sieve membranes, and STAMM discusses electroendosmose through wood membranes. Many other papers deal with the influence of electrolytes, surface phenomena, adsorption, etc., topics helpful in bringing new interpretations to life processes in plants and animals. There is a special symposium dealing with plasticity and its measurement. These colloid symposia are very helpful, and plant physiologists are welcome to the privileges of the meeting.

Back Numbers.—Attention is called again to the diminishing supply of complete sets of *PLANT PHYSIOLOGY*. The limit on the number of complete sets is set by the January number of 1926. Some of these first volumes are going to foreign subscribers, and every set sold deprives some American plant physiologist of the opportunity to own a complete file. The number remaining unsold would supply only a fraction of the demand if every physiologist desired to own a complete set of the journal.

Manuscripts.—The publication of *PLANT PHYSIOLOGY* is delayed each quarter because manuscripts are not received in sufficient number to catch up with the publication date. At the time of going to press with each number, the editors are using practically all the material on hand. Mem-

bers of the American Society of Plant Physiologists are invited to submit their best work to the journal, and to encourage others to use PLANT PHYSIOLOGY as a medium of publication of the best research. In order to catch up with the calendar we need a sufficient number of papers to make at least one additional number of 100 to 150 pages. Some of the delay can be avoided if authors will always be prompt in correcting and returning galley proofs. Those who have worth while papers rounding into shape for publication are invited to send them to some member of the editorial committee. All papers accepted will be given prompt publication, as long as the supply of papers does not exceed the funds available for their publication.

Notes.—The members of the American Society of Plant Physiologists are invited to submit brief notes concerning events of general interest, to be used in connection with this section of PLANT PHYSIOLOGY. Information regarding meetings of various kinds should be sent in several months before the meetings are held, if possible. Any other information that possesses general interest may be submitted to the editors for use in this section of the journal. These notes may be made a very helpful feature, if the members of the Society help to increase their usefulness.

The Purdue University Section.—The Purdue University Section is a very active group. Arrangements have been made to allow students to become associated with the Section and enjoy the privileges of the meetings as associate members. This arrangement should be stimulating both to the members of the Section and to the students associated with them in the meetings. The local vice president is Prof. J. F. TROSR. The meetings of the Section are delightfully informal, and the results of the meetings show that local sections are valuable to the institutions where they have been organized.

The Minnesota Section.—The local Section of the American Society of Plant Physiologists at Minnesota has made an enviable record during the current year. According to Prof. A. C. ARNY, local vice president of the Section, there are now 21 members of the Society in the Minnesota Section. That is a splendid record, and should stimulate other institutions to develop sections of the Society. The Section holds an occasional luncheon, and recently had the privilege of attending a lecture by Dr. W. J. V. OSTERHOUT in the Chemistry Auditorium. Conditions at the University of Minnesota are favorable to the development and maintenance of a virile and enthusiastic Section.

Soil Conditions and Plant Growth.—The fifth edition of this splendid monograph in the Rothamsted series on Agricultural Science has been published recently by RUSSELL. All of the chapters except the historical introduction have been rewritten, and the book enlarged about 25 per cent. in number of pages. Some changes occur in chapter headings, and one change in order of presentation. Thus, chapter IV, formerly Colloidal Properties of the Soil, is now entitled Chemical and Physical Relationships of the Soil. Chapter VII has become Chapter VI in the new edition. Other minor changes in chapter headings are noted. The book has been enormously useful, and the fifth edition will be welcomed as the best treatise on the subject available in English. The book comes from the press of Longmans, Green and Co., price \$6.50. It deserves to be in a more substantial binding.

Enzymes.—Those interested in enzymes, their properties, distribution, methods of preparation and study, and practical applications of enzyme activity, will find this book by S. A. WAKSMAN and W. C. DAVISON full of interest. It contains fifteen chapters, of which chapter VI deals with plant enzymes, and chapter VII with the enzymes of micro-organisms. Chapters X, XII, XIII, and XIV also have much for the plant physiologist, since they deal with the enzymes acting on carbohydrates, oxidizing and reducing enzymes, zymases, and catalase. The book is simply entitled *Enzymes*, and is published at \$5.50, by Williams and Wilkins, Baltimore, Maryland.

Principles of Soil Microbiology.—This book by SELMAN A. WAKSMAN, of Rutgers University, is a monumental piece of work, which presents an exhaustive survey of the field of soil microbiology. The book is divided into four sections; the first, of only one chapter, considers the occurrence and differentiation of micro-organisms in the soil. The second section, on isolation, identification and cultivation of soil micro-organisms, contains thirteen chapters each dealing with some different type of organism, ending with algae, fungi, actinomyces, protozoa, and non-protozoan fauna. The third section takes up the chemical activities of micro-organisms, their metabolism, energy transformations, decompositions of non-nitrogenous and nitrogenous material, oxidation and reduction processes, nitrogen fixation, and sulphur transformations by soil organisms. There are nine chapters in this section. The final section, soil microbiological processes and soil fertility, also contains nine chapters dealing with various phases of bacterial and other biological processes as related to the problems of plant nutrition. The final chapter in this section is a very brief orientation chapter on the history of soil microbiology, its past, present, and future. Such a book will be exceedingly useful to physiologists. The price is \$10.00, but the

book contains nearly 900 pages. The Williams and Wilkins Co. are the publishers.

New Conceptions in Colloidal Chemistry.—Under this title, E. P. Dutton and Co. announce the publication of a new book by HERBERT FREUNDLICH. The book contains nine chapters, as follows: Adsorption; the electrokinetic potential; adsorption, valency, and coagulation; the rate of coagulation; the stability of hydrophilic soils; the state of aggregation and the shape of colloidal particles; extent and change of surface in colloidal systems; photodichroism and similar phenomena. The price is \$2.00, with discounts to instructors.

PLANT PHYSIOLOGY

JULY, 1927

CARBOHYDRATE TRANSFORMATIONS IN CARROTS DURING STORAGE

HEINRICH HASSELBRING

Introduction

This paper gives the results of a study of changes taking place in carrots during storage. The changes investigated comprise the loss of water and solids and the variations in the relative proportions of sucrose, hexoses, and polysaccharides.

These changes were studied under two conditions of temperature; the one, 39° to 40° F., representing fairly well the average temperature of ordinary farm root-cellars, and the other, 32° to 35° F., approximating that usually employed in commercial cold storage for vegetables. As it was not possible, on account of the multiplicity of details, to conduct the experiments at both temperatures during one season, the experiments at the higher temperature were carried out during the season of 1924-25, and those at the lower temperature during 1925-26.

Nine varieties, representing the chief commercial types of stock and of table carrots, were studied under each temperature condition. With one exception, the same varieties were used in both cases.

The work was made possible through the cooperation of Dr. J. I. LAURITZEN, whose investigations on the effects of different storage conditions on the growth of organisms causing decay of carrots afforded an opportunity of obtaining material grown especially for the work and stored under controlled conditions.

Historical

The numerous analyses of carrots giving the proximate composition according to the Weende routine need not be especially mentioned here.

They are mostly to be found in the compilations of KÖNIG (11), JENKINS (9), JENKINS and WINTON (10), ATWATER (1), ATWATER and WOODS (2), and ATWATER and BRYANT (3), and in the reports of WERENSKIOLD (21) and of SHUTT (20). It may be of interest, however, as showing the range and variability of reducing sugars and sucrose in carrots to cite a few analyses in which these have been separately determined. As early as 1860 DIETRICH (7) gave for three varieties of carrots a dextrose content of 2.95, 8.09, and 4.84 per cent., and a corresponding sucrose content of 6.60, 3.99, and 4.16 per cent. RITTHAUSEN (17) reports analyses by FUNK showing 0.86 per cent. of crude sugar and 2.76 per cent. of dextrose in the green-shouldered white stock carrot, and 1.59 per cent. of crude sugar and 3.92 per cent. of dextrose in a red variety. According to the data given by WERENSKIOLD (21) for several varieties grown in Norway in 1894 and 1895, the reducing-sugar content ranged from 1.58 to 4.25 per cent., and the sucrose content from 1.98 to 4.94 per cent. KRISTOFFERSON (12) also found that the sucrose content and the invert-sugar content vary greatly in different varieties as well as in individual carrots of the same variety. The invert-sugar content of six varieties and strains ranged from 2.21 to 4.53 per cent., while the sucrose content recalculated from his figures varied from 2.59 to 5.81 per cent. MYERS and CROLL (14) found 6.20 per cent. of reducing-sugar and 1.30 per cent. of sucrose. It is thus apparent that there is considerable variability in the sugar content of carrots, even within the same variety.

The analyses so far reported in the literature have all been made merely from the standpoint of determining the constituents of the roots, usually without reference to previous treatment of the material. So far as I have been able to determine, no systematic analyses for the purpose of showing changes in the constituents during storage have been made.

Of the carbohydrates of the carrot, sucrose has been identified by SCHMIDT (19) and dextrose by BUSOLT (6). The occurrence of starch, as a rule, is not mentioned. Occasionally its absence is noted (19), yet NESSLER (15), according to KÖNIG, reports 0.22 per cent. of starch and BAESSLER (4) gives 0.92 per cent. for small roots and 0.87 per cent. for large ones. Details as to identification are lacking. FALK (8), also without specific identification, reports as insoluble starch the difference between the total carbohydrates after hydrolysis and the soluble carbohydrates all in terms of cuprous oxide. In the varieties used in the present investigation I have not been able to show the occurrence of granular starch. It seems, therefore, that the reducing sugars obtained by hydrolysis after extraction with 90 per cent. alcohol are derived from dextrans and hemicelluloses.

Experimental procedure

The carrots used in the present investigation were all grown in the same field on sandy loam at the Arlington experimental farm in Virginia during the summers of 1924 and 1925.

At the time of harvesting two varieties were dug each day until the harvesting was completed. The roots were washed immediately after they had been dug and were then allowed to dry over night in a cool room. On the following morning four lots of about five to six kilograms each were weighed out for each variety and put into baskets, over which heavy manila covers were then tied. One basket of each variety was immediately taken to the laboratory and prepared for analysis. The other three baskets were put into storage at the desired temperature in a room at the experimental cold storage plant at the Arlington farm. At intervals the lots of stored carrots were weighed. One basket of each variety was taken to the laboratory for analysis. The rest were carefully examined. Carrots which showed decayed spots or other signs of deterioration indicating that they might not remain in good condition over the next storage period were discarded after a record of the extent of the injuries had been made. The sound carrots remaining were reweighed and replaced in the baskets. The new weight was taken as a basis for determining the loss of weight during the next storage period. This procedure was continued until the last basket of each variety had been used for analysis.

The record of these weighings, which were made by Dr. LAURITZEN, is given in tables I and II, in order to show the actual quantities upon which the subsequent calculations and determinations were based.

The record of the extent of infection and sprouting among the stored carrots served as a basis for judging the degree to which these factors might have influenced the loss of water and solid matter from the roots. It is safe to say that this influence was practically negligible. Infected carrots occurred only sporadically, mostly during the early part of the storage season. Some varieties had no infection. As a rule, only one or two infected carrots were found in any lot, rarely more. The infections were usually limited to spots which varied from 2 to 15 millimeters in length, and only in isolated cases, when the root-tip was involved, exceeded this length. Sprouting occurred during the last storage period in a few roots of some of the varieties at the higher temperature (39° to 40° F.). The young leaves ranged from 2 to 7 centimeters in length. A comparison of the water loss from lots containing sprouted carrots with that from lots with no sprouted carrots showed no appreciable effect of the slight development of sprouts on the loss of water. The sprouted carrots were not used for analysis.

The empirical data in tables I and II do not show at a glance the comparative losses of material from the different lots of stored carrots. There-

TABLE I
WEIGHTS OF DIFFERENT LOTS OF CARROTS AT SUCCESSIVE STAGES OF STORAGE AT 39° TO 40° F.

VARIETY	AT BEGINNING OF STORAGE		AFTER 67-69 DAYS' STORAGE						AFTER 102-105 DAYS' STORAGE						AFTER 153-155 DAYS' STORAGE		
	DATE 1924	WEIGHT WHEN PLACED IN STORAGE	DATE 1925	TOTAL WEIGHT OF CARROTS	WEIGHT MINUS DISCARDED CARROTS*	LOSS OF WATER AND SOLIDS	DATE 1925	TOTAL WEIGHT OF CARROTS	WEIGHT MINUS DISCARDED CARROTS*	LOSS OF WATER AND SOLIDS	DATE 1925	TOTAL WEIGHT OF CARROTS	WEIGHT MINUS DISCARDED CARROTS*	LOSS OF WATER AND SOLIDS	DATE 1925	TOTAL WEIGHT OF CARROTS	LOSS OF WATER AND SOLIDS
Danvers Half Long	Oct. 28	6456	Jan. 5	5800	656	Feb. 9	5810†	2	1	4887	586			
"		6478		5812	666		5473	287							
"		6458		5760	698										
Blanche lisse	28	6447	5	5813	634	9	3815	205	1	2916	425			
"		6437		5775	4020	662		3341	198							
"		6433		5793	3539	640										
Jaune obtuse du Doubs	29	6495	6	5915	580	10	5132	264	2	2911	424			
"		6485		5700	5396	785		3335	224							
"		6483		5870	3559	738										
Carter's Scarlet Perfection ..	29	6490	6	5816	674	10	5404	296	2	1922	374			
"		6429		5800	629		2348	2296	157							
"		2889		2505	384											
Rouge demi-longue de Chantenay	30	6424	7	5759	665	11	4905	282	3	4820	542			
"		6493		5939	5187	554		5362	265							
"		6479		5692	5627	787										
Carter's Summer Favorite	30	6460	7	5770	690	11	5636	265	3	5088	582			
"		6482		5901	581		5670	270							
"		6500		5940	590										
Carter's Early Market	Nov. 1	6433	9	5911	522	13	5647	320	4	5007	589			
"		6482		5967	515		5596	296							
"		6488		5892	596										
Carter's Red Elephant	1	6458	9	5855	603	14	4636	299	4	4098	559			
"		5525		5927	4635	498		4657	310							
"		5588		4967	621										
Blanche à collet vert	4	6470	10	5819	651	14	5428	291	6	4804	566			
"		6447		5719	738		5370	303							
"		6490		6031	5673	459										

* Where figures appear in this column carrots were discarded, on account of infection, from the baskets continued in storage.

† This figure was not used in the calculation of the corresponding average in table III.

TABLE II
WEIGHTS OF DIFFERENT LOTS OF CARROTS AT SUCCESSIVE STAGES OF STORAGE AT 32° TO 35° F.

VARIETY	AT BEGINNING OF STORAGE		AFTER 65-68 DAYS' STORAGE				AFTER 100-103 DAYS' STORAGE				AFTER 150-155 DAYS' STORAGE			
	DATE 1925	WEIGHT WHEN PLACED IN STORAGE	DATE 1926	TOTAL WEIGHT OF CARROTS	WEIGHT MINUS DISCARDED CARROTS*	LOSS OF WATER AND SOLIDS	DATE 1926	TOTAL WEIGHT OF CARROTS	WEIGHT MINUS DISCARDED CARROTS*	LOSS OF WATER AND SOLIDS	DATE 1926	TOTAL WEIGHT OF CARROTS	WEIGHT MINUS DISCARDED CARROTS*	LOSS OF WATER AND SOLIDS
Danvers Half Long	Nov. 4	6490	Jan. 11	6255	235	Feb. 15	6055	5929	93	April 6	5874	55
"		6469		6148	321		6168	98				
Blanche lisse de la longue ..	4	6474	11	6192	5849	282	15	5721	5685	128	6	5857	128
"		6490		6151	339		6100	119				
Jaune oblique du Doubs	5	6491	12	6134	282	16	6004	126	7	5935	65
"		6438		6187	6130	251		6000	143				
Carter's Scarlet Perfection ..	5	6457	12	6192	265	16	6039	101	7	5853	124
"		6477		6140	337		5977	115				
"		6429		6092	337								
Carter's Summer Favorite	6	6418	13	6164	5881	254	17	5765	116	8	5710	117
"		6436		6191	5980	332		5827	103				
Carter's Red Elephant	7	6450	13	6112	338	17	6051	116	8	5899	81
"		6417		6107	250		5980	22				
"		6481		6257	6002	224								
Carter's Early Market	7	6493	14	6206	287	18	6056	110	9	5901	104
"		6470		6255	6166	215		6005	120				
"		6450		6125	325								
Carter's Nantes	10	6450	14	6177	273	18	6095	90	9	5709	58
"		6493		6185	308		6110	5767	117				
"		6448		6227	221								
Blanche à collet vert	10	6447	15	6230	6057	217	18	5935	122	10	5559	95
"		6483		6190	6111	191		5998	5654	113				
"		6481		6290									

* Where figures appear in this column carrots were discarded, on account of infection, from the baskets continued in storage.

fore, the figures have all been reduced to the basis of an original weight of 100 in tables III and IV. These figures show directly the shrinkage during each storage period and also permit of an immediate comparison of the shrinkage under the two storage conditions.

Other data in these tables show the percentage of water and of solids in the carrots at the different stages of storage, the actual quantities of these substances on the basis of 100 taken as the original weight of the roots, and the decrease during each storage interval in the water and solids. These data are based on averages of the figures in tables I and II, wherever more than one determination was made.

The data in tables III and IV show that at the lower storage temperature the carrots lost on the average about 7 per cent. of their weight during the entire storage season, and at the higher temperature about 26 per cent. These losses consisted mostly of water. The loss of solid matter at the lower temperature amounted to about 9.5 per cent. of the original solids, and at the higher temperature to 10.5 per cent., or about one per cent. of the total weight of the carrots. The greater part of this loss occurred during the first two months of storage. The figures relating to the loss of solids are probably not entirely accurate in detail. The positive increments (marked by a + sign in the tables) show that in a few cases the error of sampling exceeded in magnitude the loss of solid material. Nevertheless, the general agreement of the figures in each column indicates that they represent fairly well the loss through respiration.

The figures giving the differences between the water present in the roots at the beginning and at the end of each storage period show approximately the water loss during each period. To give the true loss they should be increased by the amounts representing the water formed through respiration. On the assumption that the respiratory material is glucose and that it is completely oxidized, this increase would be equal to 60 per cent. of the solid matter lost.¹

Analytical methods

(a) *Preparation of the material.*—In the preparation of the carrots for analysis, the whole of the lot except the infected and the sprouted carrots, which were discarded, was used as a sample in every case. The crowns were cut off to remove the leaf-bases. Large carrots were split once or twice, and all were cut into short pieces with a rotary slicer. The pieces were then rapidly mixed and ground through a power-driven meat grinder having a face-plate with holes 3.2 mm. in diameter. The vessel containing

¹ Theoretically, a slight further correction should be made both in the figures for the loss of solids and in the approximate figures representing the loss of water, on account of water bound by hydrolytic processes. Both errors are insignificant in the present work.

TABLE IV
SHRINKAGE, ON BASIS OF 100 AS ORIGINAL WEIGHT, OF CARROTS STORED AT 32° TO 35° F.

VARIETY	WEIGHT (EQUIVALENT TO 100), WATER CONTENT AND SOLIDS AT BEGINNING OF STORAGE				WEIGHT, WATER CONTENT AND SOLIDS AFTER 65-68 DAYS' STORAGE						WEIGHT, WATER CONTENT AND SOLIDS AFTER 100-103 DAYS' STORAGE						WEIGHT, WATER CONTENT AND SOLIDS AFTER 150-155 DAYS' STORAGE											
	DATE	ORIGINAL WEIGHT	WATER SOLIDS	WATER SOLIDS	DATE	100 SHRUNK TO	WATER SOLIDS	WATER SOLIDS	Per cent.	Actual	Actual	Decrease in Water and Solids in Carrots "A" minus "B"	DATE	100 SHRUNK TO	WATER SOLIDS	WATER SOLIDS	Per cent.	Actual	Actual	Decrease in Water and Solids in Carrots "B" minus "C"	DATE	100 SHRUNK TO	WATER SOLIDS	WATER SOLIDS	Per cent.	Actual	Actual	Decrease in Water and Solids in Carrots "C" minus "D"
Danvers Half Long	Nov. 4	100	88.74	88.74	11	95.94	88.86	85.25	3.49	15	94.25	89.10	83.98	89.10	83.98	1.27	94.02	89.25	89.25	83.91	83.91	0.07						
Blanche lisse demi-longue...	4	100	89.68	89.68	11	95.51	90.29	86.24	3.44	15	93.49	90.07	84.21	90.07	84.21	2.03	92.25	89.97	89.97	83.00	83.00	1.21						
Jaune obtuse du Doubs ...	5	100	88.57	88.57	12	95.72	88.71	84.91	3.66	16	94.21	88.71	83.57	88.71	83.57	1.34	93.28	88.98	88.98	83.00	83.00	0.57						
Scarlet's Scarlet Perfection ..	5	100	87.43	87.43	12	95.15	87.77	83.51	3.92	16	93.11	87.66	81.62	87.66	81.62	1.89	91.05	87.80	87.80	79.94	79.94	1.68						
Scarlet's Summer Favorite	6	100	89.26	89.26	13	95.72	89.65	85.81	3.45	17	93.78	89.65	84.07	89.65	84.07	1.74	91.35	89.35	89.35	81.62	81.62	2.45						
Scarlet's Red Elephant ...	7	100	87.71	87.71	13	95.80	87.74	84.05	3.66	17	95.24	87.90	83.72	87.90	83.72	0.33	94.88	88.24	88.24	83.72	83.72	0.00						
Scarlet's Early Market	7	100	88.19	88.19	14	95.74	88.19	84.43	3.76	18	94.63	88.27	83.00	88.27	83.00	0.23	91.49	88.33	88.33	80.81	80.81	2.19						
Scarlet's Nantes	10	100	88.87	88.87	14	95.87	88.60	84.94	3.93	18	94.32	88.73	83.69	88.73	83.69	1.25	93.79	88.93	88.93	83.41	83.41	0.28						
Scarlet's collet vert ..	10	100	88.97	88.97	15	96.39	89.30	86.08	2.89	18	94.41	89.20	84.21	89.20	84.21	0.30	93.65	89.21	89.21	83.55	83.55	0.66						
			11.03	11.03		10.70	10.70	10.31	0.72			10.80	10.20	10.80	10.20	0.11		10.79	10.79	10.10	10.10	0.10						

the cut pieces and the vessel receiving the pulp were kept covered with damp cloths. The pulp was rapidly mixed on a stone slab and quartered down to a workable quantity. From this were weighed out two 25-gram samples for sugar determinations, two 10-gram (25-gram in the second year's work) samples for the determination of polysaccharides, and two approximately 10-gram samples for moisture determinations. All samples were covered with neutral 95 per cent. alcohol as soon as they had been weighed out. To the beakers into which the sugar samples were weighed 0.25 of a gram of calcium carbonate had been previously added.

(b) *Determination of sugar.*—When the weighing had been completed, the sugar samples, to which enough alcohol had been added to bring the concentration to about 70 per cent., were washed into 250-ml. volumetric flasks with enough 70 per cent. alcohol to occupy about three-fourths of the volume of the flasks. The flasks were then placed in a hot water-bath and boiled for 15 minutes. Evaporation was reduced by means of empty calcium-chloride tubes loosely placed in the necks of the flasks.

The flasks containing the sugar samples were cooled, filled to the mark at 20° C. and set aside for several weeks until the determinations were begun. During that time they were frequently shaken. Since a little alcohol evaporated from the glass-stoppered flasks, they were cooled to 20° C. at intervals and refilled to the mark. After the last filling they were always allowed to stand a few days to ensure uniformity of concentration in the liquid and pulp. The extracts were subsequently treated essentially as in the alcohol extraction method described by BRYAN, GIVEN and STRAUGHN (5), 100-ml. portions being used for the operations.²

In the preparation of the alkaline tartrate solution and the copper sulphate solution, and in the reductions, the details of procedure as described by MUNSON and WALKER (13) were followed.

Beakers of approximately the same heat transmissivity were selected, according to the procedure of PETERS (16). To prevent the heating of the sides of the beakers in which the reductions were carried out, a ring of thick asbestos, cut to fit the beakers, was fastened to the asbestos gauze upon which they were heated. Before each set of determinations the flame was adjusted by trials, so as to bring the Fehling's mixture with the added sugar solution to boiling in the prescribed time. The cuprous oxide was weighed as such.

The volume occupied by the pulp, plus the undissolved calcium carbonate in the 250-ml. flasks, was determined by means of a pycnometer. Seventy per cent. alcohol of the same strength as that with which the residues had

² One ml. of a saturated solution of lead acetate $[\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}]$ was found to be suitable for clearing 100 ml. portions of these filtrates, after the necessary preliminary steps. The excess of lead was finally removed with 0.2 of a gram of dry sodium oxalate.

been previously exhaustively extracted and washed was used as a medium. The results are given in table V.

TABLE V
VOLUME OCCUPIED BY CARROT PULP

VARIETY	VOLUME OCCUPIED BY INSOLUBLE RESIDUE IN 25 GM. FRESH PULP*	VOLUME OCCUPIED BY 1 GM. OF WATER-FREE EXTRACTED RESIDUE*	VOLUME OCCUPIED BY INSOLUBLE RESIDUE IN 25 GM. FRESH PULP*	VOLUME OCCUPIED BY 1 GM. OF WATER-FREE EXTRACTED RESIDUE*
	AT BEGINNING OF STORAGE		AFTER 153-155 DAYS' STORAGE	
	ml.	ml.	ml.	ml.
Danvers Half Long	0.51	0.40	0.60	0.44
Blanche lisse demi-longue	0.57	0.53	0.55	0.46
Jaune obtuse du Doubs	0.59	0.46	0.65	0.47
Carter's Scarlet Perfection	0.62	0.44	0.82	0.50
Rouge demi- longue de Chantenay	0.53	0.44	0.55	0.44
Carter's Summer Favorite	0.46	0.40	0.49	0.41
Carter's Early Market	0.51	0.45	0.60	0.46
Carter's Red Elephant	0.70	0.48	0.84	0.50
Blanche à collet vert	0.40	0.36	0.60	0.48
Average	0.54	0.44	0.63	0.46

* Plus residual calcium carbonate.

The average volume occupied by the pulp was about 0.6 ml. A correction for this would give for the maximum percentage of reducing sugar 4.13 instead of 4.14, and for the maximum percentage of total sugar calculated as dextrose 7.24 instead of 7.26. Since the second decimal in these percentages is not significant, it was not deemed necessary to apply the correction.

(c) *Determination of acid-hydrolysable polysaccharides.*—The samples for the determination of polysaccharides insoluble in 90 per cent. alcohol and hydrolysable by 0.695 N. hydrochloric acid were stored without boiling and without calcium carbonate in flasks with 200 or 350 ml. of 95 per cent. alcohol, according as 10-gram or 25-gram samples were used. They were extracted in Soxhlet apparatus with 90 per cent. alcohol. The residues were washed into Erlenmeyer flasks with 95 per cent. alcohol which was evaporated almost to dryness in the water bath. The hydrolyses were carried out essentially according to the method of SACHSSE (18), except that for the 10-gram samples 100 ml. of acid (0.695 N.), and for the 25-gram samples 200 ml., were added to the pulp. The flasks were heated under reflux condensers for three hours in a vigorously boiling water-bath. The resulting extracts were filtered from the residues, which were washed with many small portions of hot water until, as determined by preliminary tests on collateral samples, all sugar had been removed. The filtrates, collected directly in volumetric flasks of 250-ml. or 500-ml. capacity, according as 10-gram samples or 25-gram samples had been used, were treated with one ml. of phosphotungstic acid solution (10 per cent. phosphotungstic acid in one per cent. hydrochloric acid) and, after having been cooled to 20° C., were made up to the mark. Of the solution filtered from the phosphotungstic acid precipitate, 100 ml. were nearly neutralized by the addition of 4 ml. of sodium hydroxide solution, the neutralization being finished by the addition of a trace of anhydrous sodium carbonate. After filtration, the reducing sugars were determined. The increase in volume from 100 ml. to 104 ml. was taken into consideration in the calculations of the results.

The addition of one ml. of phosphotungstic acid to 250 ml. of the solution does not have any appreciable effect on the reduction. Blanks with neutralized acid solution, to which the same quantity of phosphotungstic acid had been added, gave 0.4, 0.0, and 0.4 mg. cuprous oxide, while blanks without phosphotungstic acid gave 0.2, 0.1, and 0.2 mg. of cuprous oxide.

(d) *Determination of moisture.*—For the determination of moisture the free alcohol was evaporated from the samples at 40° to 50° C. The residues were then dried to constant weight in a slow current of air at 80° C. and at a pressure of about 8 cm. of mercury.

Analytical results

The data obtained from the analyses are given in tables VI and VII. These tables give the carbohydrate content of the roots at different stages of storage under the two temperature conditions.

The changes which take place in the carbohydrates of the carrots are, of course, not truly represented by differences in percentage composition at different stages of storage, since a direct comparison of the percentages does

TABLE VII

COMPOSITION OF CARROTS STORED AT 32° TO 35° F.

VARIETY	AT BEGINNING OF STORAGE			AFTER 65-68 DAYS' STORAGE			AFTER 100-103 DAYS' STORAGE			AFTER 150-155 DAYS' STORAGE		
	REDUC- ING SUGAR	SUCROSE	ACID HYDRO- LYSABLE POLYSAC- CHARIDES	REDUC- ING SUGAR	SUCROSE	ACID HYDRO- LYSABLE POLYSAC- CHARIDES	REDUC- ING SUGAR	SUCROSE	ACID HYDRO- LYSABLE POLYSAC- CHARIDES	REDUC- ING SUGAR	SUCROSE	ACID HYDRO- LYSABLE POLYSAC- CHARIDES
Vanvers	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Half Long	0.85	3.60	1.19	1.68	2.98	1.05	1.64	2.72	0.89	2.52	1.79	0.89
Blanche												
Blanche demi-longue	1.94	2.57	1.22	2.77	1.66	0.91	2.42	1.90	0.85	1.62	2.65	0.92
Jaune												
obtuse du Doubs	1.69	3.06	1.34	2.16	2.58	1.09	2.40	2.30	1.00	2.10	2.44	0.96
Cartier's												
Scarlet												
Perfection	1.03	4.00	1.43	1.81	2.99	1.27	1.92	2.97	1.22	2.93	1.89	1.09
Cartier's												
Summer												
Favorite	1.22	3.57	1.11	2.21	2.42	0.89	2.10	2.34	0.79	2.36	2.29	0.81
Cartier's												
Red Elephant	0.91	4.15	1.46	2.06	3.14	1.21	2.28	2.76	1.12	2.62	1.95	1.07
Cartier's												
Early												
Market	0.98	3.99	1.32	2.09	3.11	1.08	1.91	3.09	0.99	2.99	1.94	0.97
Cartier's												
Nantes	1.15	3.67	1.19	1.80	3.10	1.01	2.10	2.81	0.92	2.56	2.21	0.86
Blanche à collet vert	1.78	3.08	1.30	2.81	1.96	1.08	2.68	2.07	1.01	1.95	2.77	0.96

TABLE IX

[illegible]

not take into consideration the loss of water and other matter from the roots. In order to show the true changes, the actual quantities of the carbohydrates at the different stages have been calculated on the basis of 100 grams of the original material. These figures are given in tables VIII and IX.

Discussion

The ratio of reducing sugar to sucrose varies considerably with different varieties of carrots, and is not constant with single varieties in different years. Evidently seasonal effects on the state of development of the roots have greater influence than varietal characteristics. It can scarcely be said that any of the varieties is predominantly and constantly high either in sucrose or in dextrose. The two white varieties *Blanche lisse demi-longue* and *Blanche à collet vert*, which are regarded as stock carrots, show a somewhat lower sucrose content and a somewhat higher dextrose content than the orange varieties; but the pale yellow, *Jaune obtuse du Doubs*, grown extensively as a stock carrot, approaches the table carrots in sugar content.³

Aside from the sugars, carrots contain from about 1.25 to 1.50 per cent. of carbohydrates insoluble in 90 per cent. alcohol but easily hydrolysable by dilute acids. This material, as extraction and digestion experiments showed, consists partly of dextrin-like substances soluble in water and partly of insoluble substances hydrolysable by 0.695 N. hydrochloric acid.

The sucrose content of carrots is highest immediately after the roots have been dug and begins to decrease as soon as they have been placed in storage. The greater part of the change takes place within the first ten weeks of storage or probably within a much shorter period. Further changes in the sucrose content during the rest of the storage season are relatively small and not always in the same direction. The carrots stored at 32° to 35° F. lost, on the average, 28 per cent. of their sucrose during the first storage period and 5 and 7 per cent., respectively, during the two subsequent periods. At the higher temperature, 39° to 40° F., the average loss for all varieties during the first period was 43 per cent. of the sucrose originally present. During the next two periods there was a slight increase in sucrose in many of the varieties, resulting in an average gain of 3 and 10 per cent., respectively, for these periods. It is possible that some sucrose was reformed at this temperature.

The loss in sucrose is accompanied by a corresponding increase in reducing sugar in all varieties during the early part of the storage season. In general, there is a fair degree of proportionality between the decrease in sucrose and the increase in reducing sugar. At the higher temperature the

³ Vilmorin-Andrieux et Cie. (Les Plantes Potagères quatrième éd. p. 71) state that it is also an excellent table carrot.

increase in reducing sugar during the first storage period is followed by a loss through respiration during the rest of the season. At the lower temperature the loss becomes insignificant, some varieties even showing a small increase in reducing sugar. Too much stress should not be laid on slight differences, however, on account of the error inherent in sampling.

The dextrans and other hydrolysable carbohydrates show an average decrease during the three storage periods of 33, 8 and 7 per cent., respectively, at the higher temperature and 20, 10 and 3 per cent. at the lower. Here, as in the case of sucrose, the transformation takes place largely during the early part of the storage season. Subsequently, there is a slight but fairly consistent decrease.

Summary

Carrots stored in cold storage rooms at a temperature of 39° to 40° F. for a period of 22 weeks lost about 26 per cent. of their weight. Those stored at 32° to 35° F. for the same length of time lost about 7 per cent. The loss consists largely of water. The loss of solid matter is equal to about one per cent. of the fresh weight of the roots.

The two principal changes which take place in carrots during storage consist in a conversion of sucrose into reducing sugar, the quantity of which is correspondingly increased; and a transformation of polysaccharides to simple sugars.

Under the conditions of the experiments here reported, these processes take place more rapidly at the higher than at the lower temperature. At the higher temperature 43 per cent. of the sucrose and 33 per cent. of the polysaccharides disappear during the first ten weeks, as compared with 28 and 20 per cent. at the lower temperature. Under constant conditions, these transformations reach a sort of equilibrium during the first ten weeks of storage or sooner. The changes thereafter are small in comparison with those taking place early during storage.

Since the flavor of carrots is determined largely by their natural content of sucrose, it is evident that for canning or for cooking the quality of the roots is highest immediately after they are dug.

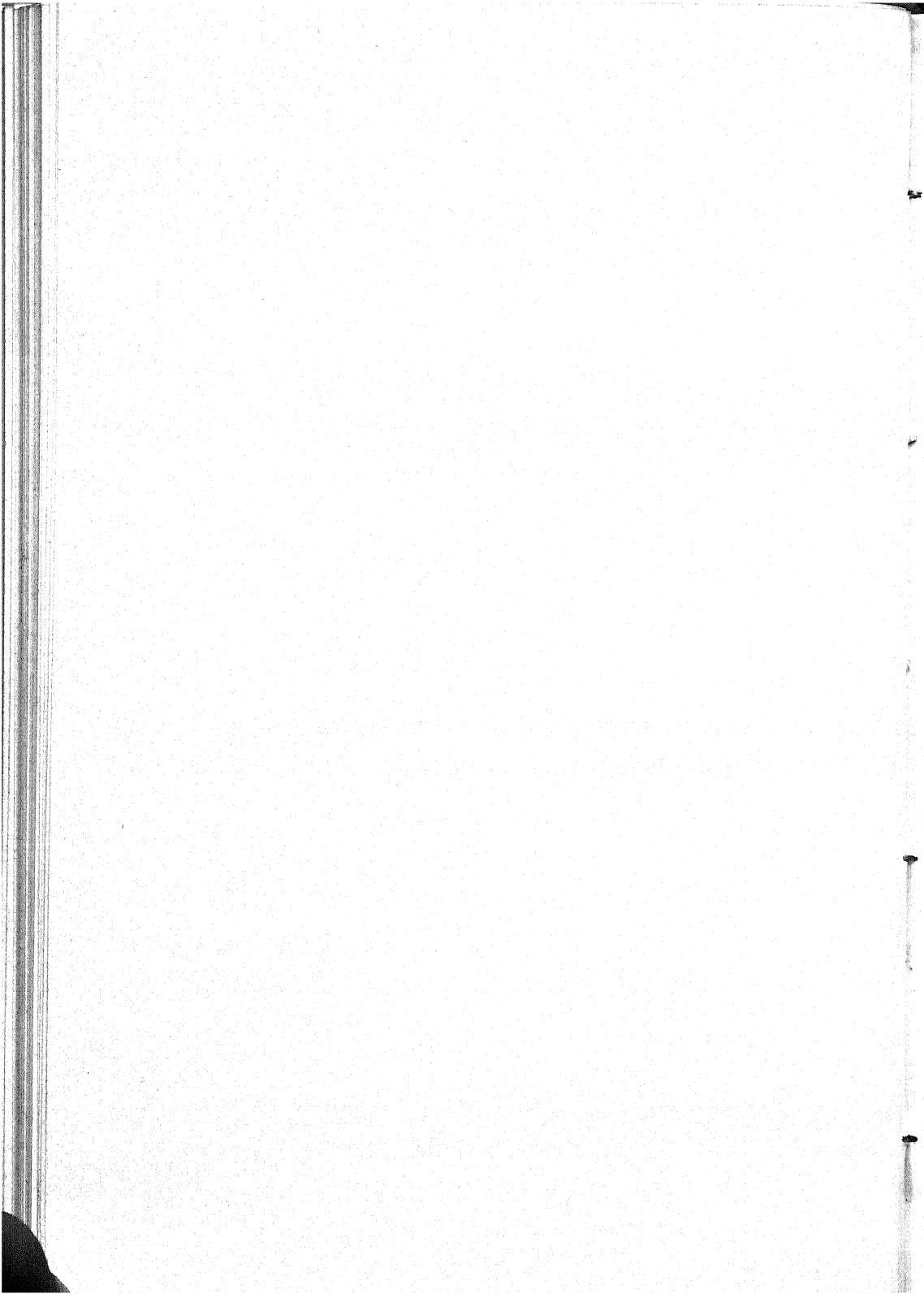
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

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NITROGENOUS METABOLISM OF *PYRUS MALUS* L.

IV. THE EFFECT OF SODIUM NITRATE APPLICATIONS ON THE TOTAL NITROGEN AND ITS PARTITION PRODUCTS IN THE LEAVES, NEW AND ONE YEAR BRANCH GROWTH THROUGHOUT A YEAR'S CYCLE¹

WALTER THOMAS

(WITH SEVEN FIGURES)

Introduction

The third paper of this series (7) gave the results of an investigation on the quantitative changes occurring in the various nitrogen fractions throughout a year's cycle in a Stayman Winesap tree, 15 years old, in the "off" year, growing in sod in the College Experimental Orchard.

Although no evidence to support the claim of certain investigators that amino-acids act as catalyzers in accelerating the rate of growth was obtained, nevertheless the results showed that amino-acids play a specific rôle. They appear to be the form in which nitrogen is carried from the roots to the metabolically active parts and, with the amides, are connected with the synthesis and utilization of proteins. Moreover, it was also shown that the unclassified or "rest" N compounds, concerning the nature of which little is known, may play a rôle as important as that of amino-acids, amines and amides, because of their apparent function in protein degradation. Finally, evidence has been produced (8) showing that the reduction of nitrates and the formation of amino-acids take place in this species for the most part in the fine roots; hence, a knowledge of the internal effects produced by nitrogenous fertilizers can only be obtained by following the fluctuation of the products or substances into which the NO_3 ion is transformed.

That a knowledge of the nitrogen distribution in the woody perennials is highly desirable is recognized by several investigators. Thus, PROEBSTING (4) has suggested that not the total nitrogen but some fraction of it might have to be considered in all attempts to correlate the relation of nutrients to the activity of the cambium. POTTER and KRAYBILL (3), discussing the behavior of bearing as compared to non-bearing spurs of apple trees with respect to the correlation between composition and spur performance, state

¹ Published with the approval of the Director of the Agricultural Experiment Station as scientific paper no. 432.

that the determination of the different forms of nitrogen is "a difficult or impossible task" but add "that if progress can be made in differentiating the forms of nitrogen available to spurs, it will be of greatest value in interpreting results." The present pioneer investigations have shown that the task is not impossible; it is, however, difficult, laborious, and time-consuming and will of necessity require a large force of workers in any attempt to correlate the performance of a large number of trees or plants receiving different fertilizer treatments with the forms of nitrogen available to the more metabolically active parts.

It is uncertain, with our present limited knowledge, to what extent the determination of the nitrogen distribution in plants can be applied to practical agriculture. Thus far, an insight has been obtained only into the quantitative changes taking place during a year's cycle of the water-soluble nitrogen fractions. The possibilities and limitations of nitrogen distribution investigations in this species are clearly defined, and more especially will they help to clarify all speculations relative to the utilization of any of the nitrogen fractions in the development of the carbohydrate-nitrogen relations in investigations relating to vegetative growth and flower formation.

Digressing for the moment, it may be pointed out here that the logical point of attacking such problems as, for example, the factors influencing fruit bud formation, which so many plant physiologists and horticulturists are at present attempting to solve, may be to ascertain first of all the internal conditions that cause differences in vigor, this being the external expression of the internal factors affecting the rate of metabolism, which, in turn, is limited by the rate of absorption of nutrients by the root system. The present partition results offer suggestions in this respect.

In the earlier metabolism investigations of the writer (6) the nitrogen partition work was carried out on one tree in the absence of the disturbing factor of developing fruit. This investigation was extended in 1924-25 to include a comparison of the course of the various nitrogen fractions throughout a year's cycle in two trees of the same variety and age growing along side one another in a homogenous soil, one of which received sodium nitrate applications and the other none. The question may be asked, what basis is there to justify a comparison of analytical results in which one tree and one tree only is compared with another of the same age and variety growing beside it in a homogenous soil? In other words, can it be postulated that the performance of the tree receiving NaNO_3 additions would have paralleled the untreated tree with which it is compared had the former received no such additions; or, if both trees had received the same nitrogen additions would the responses indicated by the analytical data be identical? From the results of investigations carried out by the writer in 1918-20, there appears to be little doubt that if the sampling is carried out on the

principles already stated (7) such comparisons as are here attempted are scientifically sound. The differences in the total nitrogen and its partition products observed in the present investigation, therefore, can logically be attributed to the addition of sodium nitrate to the one tree and not to the other.

Materials and methods

Two Stayman Winesap trees, 16 years old, growing adjacent to one another in the College Orchard, both of which had always been subjected to the same general treatment, and which resulted in very similar growth and reproductive responses, were used in this investigation. One of these trees, no. E-20, was treated with 10 pounds of sodium nitrate on April 20, 1924, just after the buds were commencing to swell, and again with another 10 pounds on June 8, 1924, at the initiation of fruit bud formation. This quantity is four times the application normally given, the object being to accentuate whatever internal differences might be produced in the nitrogen metabolism of the trees by the nitrate additions as indicated by the partition results. The external differences that existed were shown by the much darker color of the foliage and greater vegetative extension of the treated tree. Reproduction, as indicated by the yields, was 8 per cent. less on the treated tree, which would lead to the conclusion that if the theory of carbohydrate-nitrogen ratios holds, the excessive nitrate applications have tended to shift the treated trees from class III to class II in KRAUS and KRAYBILL's classification.

The collection of samples was carried out under favorable meteorological conditions in the early morning, in the manner already described (7). The collection was made on ten different dates between April 4, 1924, and November 11, 1924, the samples being taken to the laboratory immediately and dried in the manner already indicated (6).

As in the earlier experiments, no correlation could be found between the total nitrogen or any of its fractions and any of the climatic factors. However, a summary of the weather conditions is appended as a matter of record.

THE WOOD AND BARK SEPARATION PROBLEM.—In all collections dating from June 11, 1924, the wood and bark of the branch growths were separated before preserving the samples by desiccation, but, as pointed out in the third paper (7), it was found that no special advantage is to be derived from this procedure and that, moreover, the interpretation of the nitrogen partition results is not affected by such separations. Also, owing to the relatively small quantity of each type of branch tissues available for the partition work, it was found in many cases that there was either insufficient "wood" or "bark" to carry out satisfactorily the analytical work upon them separately. Most of the work, therefore, was carried out on samples of

TABLE I
SUMMARY OF METEOROLOGICAL DATA

DATES	MEAN MAXIMUM	MEAN MINIMUM	RAINFALL	SUNSHINE	HUMIDITY
1924	Deg. F.	Deg. F.	Inches	Per cent.	Per cent.
April 4-April 21.....	53.5	34.8	3.26	59.4	68.7
April 22-April 28.....	62.3	36.7	0.28	83.3	63.9
April 29-May 13.....	62.5	43.5	4.65	54.0	79.2
May 14-May 22.....	59.9	43.0	1.33	41.9	76.5
May 23-June 11.....	63.2	45.9	2.35	48.9	77.0
June 12-July 16.....	76.7	56.0	7.07	64.7	79.3
July 17-August 28.....	78.4	56.5	4.30	76.3	76.3
August 29-October 7.....	68.5	46.9	4.54	42.9	79.8
October 8-November 11.....	60.5	37.2	0.16	69.6

“wood” and “bark” combined in the proportions in which they were present in the original sample. There is, therefore, as far as the principal nitrogen fractions are concerned, little danger of a deficiency due to localization of the metabolically active nitrogen fractions in some tissues at the expense of others. Consequently, all analytical data have been re-calculated to the original basis as if wood and bark had been ground up together.

THE METHOD IN DETAIL.—The extractions with water were carried out in the manner outlined in the first paper (6).

The two fractionation schemes adopted in these metabolism investigations have already been discussed in detail (7). The second fractionation scheme was adopted in the present work, as fewer separations are involved, a weighty consideration where a large number of samples is to be examined, in spite of the fact that a consideration of the accuracy and limits of error as given in table III indicates that this second scheme is subject to greater analytical errors than the first scheme. However, for the present comparative studies the second scheme serves quite well.

In the non-protein filtrates from the colloidal ferric hydroxide precipitation (6), the following fractions were determined:—Ammonia N, amide N (asparagine and possibly glutamine N), basic N, α -mono-amino N, and also humin and melanin N from the amide and basic nitrogen determinations. The sum of these nitrogen fractions subtracted from the non-protein nitrogen gives the “rest” nitrogen.

1. *Hygroscopic water*.—This was determined on a two gram sample dried at 110° C. for 24 hours.

2. *Total water-soluble N*.—This fraction was determined in the usual way by the Kjeldahl method on 200 cc. aliquots, adopting standard methods and precautions for estimating small quantities of nitrogen.

3. *Ammonia N*.—The ammonia N was determined on a 500 cc. aliquot before treatment with colloidal iron and for reasons already given (7).

4. *Non-protein N*.—This was determined in the same manner as the total water-soluble nitrogen on 200 cc. aliquots of the filtrates from the colloidal ferric hydroxide precipitation.

5. *Amide N (asparagine and possibly glutamine N)*.—Up to this stage the fractionation method is similar to that adopted in the earlier investigations (7); the point of departure is made at this stage. The determination of amide N is carried out essentially by SACHSSE's method (5), *i.e.*, by adding a definite quantity of HCl (sufficient to give a 4 per cent. solution) to the whole of the non-protein N filtrate remaining after the removal of the aliquots for the determination of nitrogen (which usually amounts to 1,600 cc.), and then hydrolyzing for three hours. The solution remaining after the removal of the HCl *in vacuo* is diluted to about 300 cc. with ammonia-free distilled water and distilled *in vacuo* with solid CaO (7). The asparagine N (with possibly some glutamine N) is obtained by subtracting the free ammonia, determined as previously indicated, from the amount obtained in this determination, *i.e.*, after hydrolysis. Proteoses were present in too small a quantity to affect the amide N results.

6. *Humins N and Ca(OH)₂ melanin N and basic N*.—The solution remaining in the flask from the amide N determination is filtered from the humins and Ca(OH)₂ melanin N, washed with hot water until free from chlorides, and the nitrogen content of these precipitates determined.

7. *The basic N*.—HAUSMANN's method (1) with modifications (2, 9) as indicated later was adopted. The filtrate and washings were concentrated *in vacuo* to about 80 cc. and then transferred to a beaker, the contents being cooled to 20° C. Afterwards 2.5 cc. concentrated H₂SO₄ were slowly added, followed by a solution containing 20 gm. of phosphotungstic acid and 5 gm. concentrated H₂SO₄ per 100 cc., the mixture being added drop by drop. Six cc. of this phosphotungstic acid mixture were quite sufficient in the present work. Care is to be taken to avoid more than a slight excess of the phosphotungstic acid solution; otherwise difficulty will be encountered in the subsequent amino-acid determination, owing to precipitation of some of the phosphotungstic acid. The solutions were diluted to 200 cc., heated on the water bath until the precipitates of the bases were dissolved, and then allowed to stand for two or three days. The granular precipitate was filtered off and washed with a small quantity of phosphotungstic acid solution containing 2.5 gm. phosphotungstic acid and 5 gm. concentrated H₂SO₄ per 100 cc. The technique of washing these precipitates was carried out in accordance with OSBORNE and HARRIS's (2) recommendations. VAN SLYKE (9) gives additional observations on the care in precipitating and washing

TABLE II
THE PERCENTAGES OF IMBIBITIONAL AND TOTAL WATER
ONE YEAR BRANCH GROWTH (TREE NO. E-20)

SERIES NO.	COLLECTING DATE	DESCRIPTION	FRESH WEIGHT	MOISTURE FREE WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
			gm.	gm.	Per cent.	Per cent.
76	April 4, 1924.....	(wood and bark)	70.0	35.7	49.0	58.1
77	April 21, 1924.....	(wood and bark)	121.0	58.1	52.0	59.0
92	April 28, 1924.....	(wood and bark)	55.0	27.0	50.9	56.9
114	May 13, 1924.....	(wood and bark)	73.0	35.0	52.0	58.4
142	May 22, 1924.....	(wood and bark)	51.0	22.0	56.9	57.8
161	June 11, 1924.....	(wood)	55.5	28.8	48.1	51.7
162	June 11, 1924.....	(bark)	43.8	17.9	59.1	60.7
185A	July 16, 1924.....	(wood)	78.8	43.7	44.4	46.8
185B	July 16, 1924.....	(bark)	56.6	22.6	58.8	61.8
196	August 28, 1924.....	(wood)	70.1	39.5	43.7	46.9
196A	August 28, 1924.....	(bark)	52.6	22.6	57.0	60.9
203A	October 7, 1924.....	(wood)	88.3	55.6	37.0	45.0
203B	October 7, 1924.....	(bark)	63.1	28.2	55.3	57.3
237	November 11, 1924	(wood)	123.0	71.1	42.2	44.7
238	November 11, 1924	(bark)	78.4	38.4	50.8	54.0

ONE YEAR BRANCH GROWTH (TREE NO. E-22)

SERIES NO.	COLLECTING DATE	DESCRIPTION	FRESH WEIGHT	MOISTURE FREE WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
			gm.	gm.	Per cent.	Per cent.
60	April 4, 1924.....	(wood and bark)	69.0	35.0	49.3	53.0
68	April 21, 1924.....	(wood and bark)	118.0	56.0	52.5	56.2
86A	April 28, 1924.....	(wood and bark)	69.0	34.0	50.7	60.0
105	May 13, 1924.....	(wood and bark)	81.0	37.0	54.3	60.4
124	May 22, 1924.....	(wood and bark)	53.3	21.6	59.4	60.0
154	June 11, 1924.....	(wood)	52.5	33.0	37.7	42.9
155	June 11, 1924.....	(bark)	54.0	24.3	55.0	60.2
180A	July 16, 1924.....	(wood)	72.4	41.0	43.6	47.8
180B	July 16, 1924.....	(bark)	51.8	21.4	56.4	60.9
199A	August 28, 1924.....	(wood)	71.2	40.4	43.2	44.2
199B	August 28, 1924.....	(bark)	53.6	26.7	47.5	58.7
215A	October 7, 1924.....	(wood)	90.6	54.4	40.0	43.1
215B	October 7, 1924.....	(bark)	65.0	36.5	56.2	57.4
218	November 11, 1924	(wood)	120.0	42.4	35.4	40.6
217	November 11, 1924	(bark)	75.0	33.7	52.2	57.0

TABLE II (*Continued*)
THE PERCENTAGES OF IMBIBITIONAL AND TOTAL WATER
NEW GROWTH (TREE NO. E-20)

SERIES NO.	COLLECTING DATE	DESCRIPTION	FRESH WEIGHT	MOIS- TURE FREE WEIGHT	IMBIBI- TIONAL WATER	TOTAL WATER
			gm.	gm.	Per cent.	Per cent.
160	June 11, 1924.....		50.0	14.4	66.2	71.2
185	July 16, 1924.....	(wood)	32.0	12.0	62.5	65.4
186	July 16, 1924.....	(bark)	46.0	16.0	65.2	68.1
191	August 28, 1924.....	(wood)	30.0	16.0	46.6	50.2
192	August 28, 1924.....	(bark)	31.0	14.0	54.8	57.9
206	October 7, 1924.....	(wood)	52.3	31.0	40.7	44.1
207	October 7, 1924.....	(bark)	66.6	21.5	67.7	69.8
217	November 11, 1924	(wood)	68.0	42.5	37.5	43.9
218	November 11, 1924	(bark)	51.0	31.2	38.8	44.5

NEW GROWTH (TREE NO. E-22)

SERIES NO.	COLLECTING DATE	DESCRIPTION	FRESH WEIGHT	MOIS- TURE FREE WEIGHT	IMBIBI- TIONAL WATER	WATER TOTAL
			gm.	gm.	Per cent.	Per cent.
153	June 11, 1924.....		64.0	20.8	67.5	72.9
188A	July 16, 1924.....	(wood)	31.6	12.3	60.8	64.2
189	July 16, 1924.....	(bark)	46.7	17.7	61.8	65.6
196A	August 28, 1924.....	(wood)	37.0	21.0	43.2	47.3
196	August 28, 1924.....	(bark)	40.0	19.0	52.5	54.2
212	October 7, 1924.....	(wood)	46.9	29.0	38.0	42.0
211	October 7, 1924.....	(bark)	40.5	21.1	48.1	51.6
230	November 11, 1924	(wood)	62.6	38.0	37.7	41.9
231	November 11, 1924	(bark)	54.2	27.6	49.1	52.6

these phosphotungstic acid precipitates. However, no special difficulty was encountered in washing them. Since the precipitates were small, care had to be exercised to employ small filters and to avoid more than two or three washings, using suction and a wash solution cooled to 0° C. In this respect the technique differs from that employed in protein work, in which the quantities of basic nitrogen obtained from 2 to 3 gm. of protein are comparatively large.

Nitrogen was determined in these precipitates in the usual way, the precipitate and filter being transferred to the Kjeldahl flash direct, since it was not necessary to decompose the basic phosphotungstic precipitates, inas-

TABLE II (*Concluded*)

THE PERCENTAGES OF IMBIBITIONAL AND TOTAL WATER

LEAVES (TREE NO. E-20)

SERIES NO.	COLLECTING DATE	FRESH WEIGHT	MOISTURE FREE WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
		gm.	gm.	Per cent.	Per cent.
138	May 13, 1924	50.0	13.0	74.0	77.0
140	May 22, 1924	70.3	20.0	72.6	76.4
166	June 11, 1924	97.0	32.0	67.0	70.0
189	July 16, 1924	204.0	82.0	59.8	62.6
195	August 28, 1924	144.0	60.0	58.3	60.7
210	October 7, 1924	170.0	77.0	54.7	57.9
222A	November 11, 1924	40.0	19.3	51.8	56.3

LEAVES (TREE NO. E-22)

SERIES NO.	COLLECTING DATE	FRESH WEIGHT	MOISTURE FREE WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
		gm.	gm.	Per cent.	Per cent.
106	May 13, 1924	49.0	16.0	66.7	72.3
129	May 22, 1924	73.3	18.0	75.4	77.0
153A	June 11, 1924	85.0	24.3	60.4	65.6
180	July 16, 1924	203.0	82.0	59.6	65.6
199	August 28, 1924	214.0	89.0	58.4	61.4
215	October 7, 1924	168.0	63.5	62.2	65.2
233A	November 11, 1924	38.0	15.5	59.2	64.3

much as the determination of the diamino acids—histidine, arginine and lysine—was not under consideration in this investigation.

8. *α-Mono-amino N*.—The filtrate and washings from the phosphotungstic acid precipitation were made alkaline (pH 7.5) with 50 per cent. NaOH and then acid (pH 6.0) with acetic acid, concentrated *in vacuo* to about 80 cc., *i.e.*, to the point at which salts begin to separate out, and finally made up to a definite volume (usually 100 cc.). The *α-mono-amino N* was determined in the usual way in the VAN SLYKE micro-apparatus.

9. "*Rest*" *N*.—This was calculated by difference, as already described.

Experimental results

A description of the samples with their fresh and moisture free weights is given in table II, and the partition results in table III.

TABLE III

L NITROGEN AND TOTAL WATER-SOLUBLE NITROGEN IN THE NEW (1924) BRANCH GROWTH, THE ONE YEAR (1923) BRANCH GROWTH AND LEAVES, TOGETHER WITH THE DISTRIBUTION OF THE NON-PROTEIN CONSTITUENTS EXPRESSED AS PERCENTAGES OF FRESH AND MOISTURE FREE WEIGHTS, RESPECTIVELY
ONE YEAR BRANCH GROWTH (TREE NO. E-20)

IS	COLLECTING DATE	TOTAL N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	AMMONIA N	AMINO N	AMIDE N	BASIC N	HUMIN N	RESIDUAL N
					As percentages of fresh weight of material					
	April 4, 1924.....	0.263	0.0370	0.0317	0.0059	0.0041	0.0029	0.0046	0.0015	0.0126
A	April 21, 1924.....	0.308	0.0463	0.0380	0.0030	0.0070	0.0094	0.0057	0.0028	0.0197
B	April 26, 1924.....	0.329	0.0698	0.0541	0.0022	0.0084	0.0086	0.0108	0.0028	0.0216
A	May 13, 1924.....	0.225	0.0582	0.0458	0.0012	0.0064	0.0042	0.0083	0.0027	0.0229
B	May 22, 1924.....	0.287	0.0789	0.0429	0.0019	0.0120	0.0080	0.0096	0.0029	0.0084
	June 11, 1924.....	0.248	0.0443	0.0349	0.0013	0.0035	0.0049	0.0066	0.0026	0.0159
A	July 16, 1924.....	0.310	0.0733	0.0455	0.0014	0.0113	0.0127	0.0042	0.0035	0.0122
B	August 28, 1924.....	0.274	0.0643	0.0531	0.0020	0.0070	0.0110	0.0107	0.0021	0.0201
A	October 7, 1924.....	0.298	0.0697	0.0598	0.0017	0.0080	0.0134	0.0099	0.0027	0.0239
B	November 11, 1924.....	0.346	0.0852	0.0716	0.0020	0.0130	0.0140	0.0110	0.0030	0.0275
					As percentages of moisture free weight of material					
	April 4, 1924.....	0.628	0.0835	0.0756	0.0140	0.0098	0.0070	0.0110	0.0035	0.0303
A	April 21, 1924.....	0.750	0.1130	0.0929	0.0012	0.0170	0.0230	0.0140	0.0068	0.0481
B	April 26, 1924.....	0.764	0.1620	0.1256	0.0050	0.0195	0.0200	0.0250	0.0066	0.0500
A	May 13, 1924.....	0.540	0.1400	0.1101	0.0030	0.0155	0.0100	0.0200	0.0066	0.0550
B	May 22, 1924.....	0.680	0.1870	0.1017	0.0045	0.0285	0.0190	0.0228	0.0069	0.0200
	June 11, 1924.....	0.560	0.1000	0.0788	0.0030	0.0080	0.0110	0.0150	0.0058	0.0360
A	July 16, 1924.....	0.660	0.1560	0.0968	0.0030	0.0240	0.0270	0.0090	0.0075	0.0260
B	August 28, 1924.....	0.545	0.1280	0.1057	0.0040	0.0140	0.0220	0.0214	0.0042	0.0400
A	October 7, 1924.....	0.600	0.1400	0.1200	0.0035	0.0160	0.0270	0.0200	0.0055	0.0480
B	November 11, 1924.....	0.690	0.1700	0.1430	0.0040	0.0260	0.0280	0.0220	0.0060	0.0550

TABLE II (*Concluded*)

THE PERCENTAGES OF IMBIBITIONAL AND TOTAL WATER
LEAVES (TREE NO. E-20)

SERIES NO.	COLLECTING DATE	FRESH WEIGHT	MOISTURE FREE WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
		gm.	gm.	Per cent.	Per cent.
138	May 13, 1924	50.0	13.0	74.0	77.0
140	May 22, 1924	70.3	20.0	72.6	76.4
166	June 11, 1924	97.0	32.0	67.0	70.0
189	July 16, 1924	204.0	82.0	59.8	62.6
195	August 28, 1924	144.0	60.0	58.3	60.7
210	October 7, 1924	170.0	77.0	54.7	57.9
222A	November 11, 1924	40.0	19.3	51.8	56.3

LEAVES (TREE NO. E-22)

SERIES NO.	COLLECTING DATE	FRESH WEIGHT	MOISTURE FREE WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
		gm.	gm.	Per cent.	Per cent.
106	May 13, 1924	49.0	16.0	66.7	72.3
129	May 22, 1924	73.3	18.0	75.4	77.0
153A	June 11, 1924	85.0	24.3	60.4	65.6
180	July 16, 1924	203.0	82.0	59.6	65.6
199	August 28, 1924	214.0	89.0	58.4	61.4
215	October 7, 1924	168.0	63.5	62.2	65.2
233A	November 11, 1924	38.0	15.5	59.2	64.3

much as the determination of the diamino acids—histidine, arginine and lysine—was not under consideration in this investigation.

8. *α-Mono-amino N*.—The filtrate and washings from the phosphotungstic acid precipitation were made alkaline (pH 7.5) with 50 per cent. NaOH and then acid (pH 6.0) with acetic acid, concentrated *in vacuo* to about 80 cc., *i.e.*, to the point at which salts begin to separate out, and finally made up to a definite volume (usually 100 cc.). The *α-mono-amino N* was determined in the usual way in the VAN SLYKE micro-apparatus.

9. "*Rest*" *N*.—This was calculated by difference, as already described.

Experimental results

A description of the samples with their fresh and moisture free weights is given in table II, and the partition results in table III.

TABLE III

TOTAL NITROGEN AND TOTAL WATER-SOLUBLE NITROGEN IN THE NEW (1924) BRANCH GROWTH, THE ONE YEAR (1923) BRANCH GROWTH AND LEAVES, TOGETHER WITH THE DISTRIBUTION OF THE NON-PROTEIN CONSTITUENTS EXPRESSED AS PERCENTAGES OF FRESH AND MOISTURE FREE WEIGHTS, RESPECTIVELY
 ONE YEAR BRANCH GROWTH (TREE NO. E-20)

SERIES NO.	COLLECTING DATE	TOTAL N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	AMMONIA N	AMINO N	AMIDE N	BASIC N	HUMIN N	RESIDUAL N
			As percentages of fresh weight of material							
76	April 4, 1924	0.263	0.0370	0.0317	0.0059	0.0041	0.0029	0.0046	0.0015	0.0126
77	April 21, 1924	0.308	0.0463	0.0340	0.0005	0.0070	0.0094	0.0057	0.0028	0.0197
92	April 28, 1924	0.329	0.0698	0.0581	0.0022	0.0084	0.0086	0.0108	0.0028	0.0216
14	May 13, 1924	0.225	0.0582	0.0458	0.0012	0.0064	0.0042	0.0083	0.0027	0.0229
42	May 22, 1924	0.287	0.0789	0.0429	0.0019	0.0120	0.0080	0.0096	0.0029	0.0084
61										
62	June 11, 1924	0.248	0.0443	0.0349	0.0013	0.0035	0.0049	0.0066	0.0026	0.0159
85A										
85B	July 16, 1924	0.310	0.0733	0.0455	0.0014	0.0113	0.0127	0.0042	0.0035	0.0122
96	August 28, 1924	0.274	0.0643	0.0531	0.0020	0.0070	0.0110	0.0107	0.0021	0.0201
703A										
337	October 7, 1924	0.298	0.0697	0.0598	0.0017	0.0080	0.0134	0.0099	0.0027	0.0239
738	November 11, 1924	0.346	0.0852	0.0716	0.0020	0.0130	0.0140	0.0110	0.0030	0.0275
			As percentages of moisture free weight of material							
76	April 4, 1924	0.628	0.0835	0.0756	0.0140	0.0098	0.0070	0.0110	0.0035	0.0303
77	April 21, 1924	0.750	0.1130	0.0929	0.0012	0.0170	0.0230	0.0140	0.0068	0.0481
92	April 28, 1924	0.764	0.1620	0.1256	0.0050	0.0195	0.0200	0.0250	0.0066	0.0500
14	May 13, 1924	0.540	0.1400	0.1101	0.0030	0.0155	0.0100	0.0200	0.0066	0.0550
42	May 22, 1924	0.680	0.1870	0.1017	0.0045	0.0285	0.0190	0.0228	0.0069	0.0200
61										
62	June 11, 1924	0.560	0.1000	0.0788	0.0030	0.0080	0.0110	0.0150	0.0058	0.0360
85A										
85B	July 16, 1924	0.660	0.1560	0.0968	0.0030	0.0240	0.0270	0.0090	0.0075	0.0260
96	August 28, 1924	0.545	0.1280	0.1057	0.0040	0.0140	0.0220	0.0214	0.0042	0.0400
703A										
703B	October 7, 1924	0.600	0.1400	0.1200	0.0035	0.0160	0.0270	0.0200	0.0055	0.0480
337										
738	November 11, 1924	0.690	0.1700	0.1430	0.0040	0.0260	0.0280	0.0220	0.0060	0.0550

TABLE III (Continued)

TOTAL NITROGEN AND TOTAL WATER-SOLUBLE NITROGEN IN THE NEW (1924) BRANCH GROWTH, THE ONE YEAR (1923) BRANCH GROWTH AND LEAVES, TOGETHER WITH THE DISTRIBUTION OF THE NON-PROTEIN CONSTITUENTS EXPRESSED AS PERCENTAGES OF FRESH AND MOISTURE FREE WEIGHTS, RESPECTIVELY
ONE YEAR BRANCH GROWTH (TREE NO. E-22)

SERIES NO.	COLLECTING DATE	TOTAL N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	AMMONIA N	AMINO N	AMIDE N	BASIC N	HUMIN N	RESIDUAL N
			As percentages of fresh weight of material							
60	April 4, 1924.....	0.299	0.0384	0.0286	0.0086	0.0045	0.0034	0.0047	0.0010	0.0086
68	April 21, 1924.....	0.279	0.0520	0.0389	0.0026	0.0068	0.0057	0.0064	0.0013	0.0160
86A	April 28, 1924.....	0.288	0.0560	0.0395	0.0022	0.0076	0.0047	0.0058	0.0032	0.0160
105	May 13, 1924.....	0.166	0.0416	0.0309	0.0016	0.0031	0.0014	0.0041	0.0030	0.0168
124	May 22, 1924.....	0.186	0.0385	0.0375
154	June 11, 1924.....	0.171	0.0353	0.0290	0.0015	0.0013	0.0027	0.0030	0.0036	0.0169
180A	July 16, 1924.....	0.153	0.0387	0.0257	0.0016	0.0025	0.0025	0.0027	0.0036	0.0128
199A	August 28, 1924.....	0.158	0.0321	0.0211	0.0017	0.0015	0.0048	0.0032	0.0025	0.0075
215A	October 7, 1924.....	0.151	0.0357	0.0262	0.0014	0.0028	0.0061	0.0034	0.0029	0.0096
215B	November 11, 1924.....	0.208	0.0432	0.0352	0.0019	0.0045	0.0071	0.0040	0.0024	0.0152
228			As percentages of moisture free weight of material							
229										
60	April 4, 1924.....	0.635	0.0818	0.0608	0.0140	0.0095	0.0072	0.0100	0.0021	0.0182
68	April 21, 1924.....	0.638	0.1187	0.0887	0.0060	0.0155	0.0130	0.0147	0.0030	0.0365
86A	April 28, 1924.....	0.720	0.1400	0.0988	0.0055	0.0190	0.0118	0.0145	0.0080	0.0400
105	May 13, 1924.....	0.480	0.1203	0.0893	0.0046	0.0090	0.0040	0.0118	0.0088	0.0485
124	May 22, 1924.....	0.465	0.1051	0.0865	0.0040	0.0120	0.0085	0.0165	0.0080	0.0400
154	June 11, 1924.....	0.405	0.0832	0.0687	0.0035	0.0030	0.0065	0.0072	0.0085	0.0400
155	July 16, 1924.....	0.340	0.0860	0.0570	0.0035	0.0055	0.0035	0.0060	0.0080	0.0285
180B	August 28, 1924.....	0.315	0.0640	0.0422	0.0034	0.0030	0.0095	0.0063	0.0050	0.0150
199B	October 7, 1924.....	0.314	0.0740	0.0543	0.0030	0.0058	0.0126	0.0070	0.0060	0.0200
215A	November 11, 1924.....	0.437	0.0910	0.0740	0.0040	0.0095	0.0150	0.0085	0.0050	0.0320

TABLE III (Continued)

TOTAL NITROGEN AND TOTAL WATER-SOLUBLE NITROGEN IN THE NEW (1924) BRANCH GROWTH, THE ONE YEAR (1923) BRANCH GROWTH AND LEAVES, TOGETHER WITH THE DISTRIBUTION OF THE NON-PROTEIN CONSTITUENTS EXPRESSED AS PERCENTAGES OF FRESH AND MOISTURE FREE WEIGHTS, RESPECTIVELY
NEW GROWTH (TREE NO. E-20)

SERIES NO.	COLLECTING DATE	TOTAL N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	AMMONIA N	AMINO N	AMIDE N	BASIC N	HUMIN N	RESIDUAL N
					As percentages of fresh weight of material					
160	June 11, 1924.....	0.432	0.0490	0.0369	0.0013	0.0124	0.0078	0.0046	0.0013	0.0092
185										
186	July 16, 1924.....	0.363	0.0818	0.0714	0.0035	0.0250	0.0089	0.0073	0.0035	0.0228
191										
192	August 28, 1924.....	0.378	0.0771	0.0716	0.0010	0.0207	0.0128	0.0096	0.0034	0.0246
206										
207	October 7, 1924.....	0.424	0.0979	0.0810	0.0031	0.0265	0.0148	0.0069	0.0032	0.0278
217										
218	November 11, 1924..	0.517	0.1114	0.0964	0.0023	0.0281	0.0201	0.0046	0.0040	0.0373
					As percentages of moisture free weight of material					
160	June 11, 1924.....	1.503	0.1700	0.1290	0.0045	0.0430	0.0270	0.0160	0.0045	0.0320
185										
186	July 16, 1924.....	0.843	0.1900	0.1650	0.0082	0.0580	0.0206	0.0170	0.0082	0.0530
191										
192	August 28, 1924.....	0.784	0.1600	0.1485	0.0020	0.0430	0.0265	0.0200	0.0070	0.0510
206										
207	October 7, 1924.....	0.802	0.1850	0.1533	0.0058	0.0500	0.0280	0.0130	0.0060	0.0525
217										
218	November 11, 1924..	0.900	0.1940	0.1680	0.0040	0.0490	0.0350	0.0080	0.0070	0.0650

TABLE III (Continued)

TOTAL NITROGEN AND TOTAL WATER-SOLUBLE NITROGEN IN THE NEW (1924) BRANCH GROWTH, THE ONE YEAR (1923) BRANCH GROWTH AND LEAVES, TOGETHER WITH THE DISTRIBUTION OF THE NON-PROTEIN CONSTITUENTS EXPRESSED AS PERCENTAGES OF FRESH AND MOISTURE FREE WEIGHTS, RESPECTIVELY
NEW GROWTH (TREE NO. E-22)

SERIES NO.	COLLECTING DATE	TOTAL N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	AMMONIA N	AMINO N	AMIDE N	BASIC N	HUMIN N	RESIDUAL N
			As percentages of fresh weight of material							
153	June 11, 1924.....	0.379	0.0278	0.0215	0.0012	0.0063	0.0033	0.0028	0.0023	0.0057
181	July 16, 1924.....	0.199	0.0378	0.0309	0.0013	0.0079	0.0031	0.0046	0.0015	0.0125
182	August 28, 1924.....	0.227	0.0414	0.0330	0.0019	0.0062	0.0041	0.0036	0.0027	0.0145
196A	October 7, 1924.....	0.276	0.0500	0.0377	0.0017	0.0062	0.0070	0.0037	0.0040	0.0193
196B	November 11, 1924..	0.300	0.0527	0.0462	0.0013	0.0073	0.0087	0.0031	0.0029	0.0213
211			As percentages of moisture free weight of material							
212	June 11, 1924.....	1.400	0.1026	0.0703	0.0043	0.0230	0.0120	0.0102	0.0086	0.0212
230	July 16, 1924.....	0.605	0.1150	0.0940	0.0040	0.0240	0.0095	0.0140	0.0045	0.0380
231	August 28, 1924.....	0.547	0.1000	0.0798	0.0045	0.0150	0.0100	0.0088	0.0065	0.0350
153	October 7, 1924.....	0.557	0.1010	0.0762	0.0035	0.0125	0.0142	0.0075	0.0080	0.0390
181	November 11, 1924..	0.603	0.1089	0.0954	0.0030	0.0150	0.0180	0.0064	0.0060	0.0440

TABLE III (Continued)

TOTAL NITROGEN AND TOTAL WATER-SOLUBLE NITROGEN IN THE NEW (1924) BRANCH GROWTH, THE ONE YEAR (1923) BRANCH GROWTH AND LEAVES, TOGETHER WITH THE DISTRIBUTION OF THE NON-PROTEIN CONSTITUENTS EXPRESSED AS PERCENTAGES OF FRESH AND MOISTURE FREE WEIGHTS, RESPECTIVELY
LEAVES (TREE NO. E-22)

SERIES NO.	COLLECTING DATE	TOTAL N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	AMMONIA N	AMINO N	AMIDE N	BASIC N	HUMIN N	RESIDUAL N
						As percentages of fresh weight of material				
138	May 13, 1924	0.830	0.0690	0.0575	0.0019	0.0092	0.0028	0.0120	0.0081	0.0219
140	May 22, 1924	0.806	0.0802	0.0658	0.0015	0.0145	0.0042	0.0165	0.0078	0.0217
166	June 11, 1924	0.774	0.0732	0.0660	0.0012	0.0138	0.0027	0.0165	0.0063	0.0219
189	July 16, 1924	0.804	0.1058	0.0954	0.0022	0.0204	0.0026	0.0224	0.0120	0.0370
195	August 28, 1924	0.817	0.1014	0.0880	0.0016	0.0137	0.0076	0.0157	0.0110	0.0385
210	October 7, 1924	0.777	0.0973	0.0869	None	0.0147	0.0084	0.0177	0.0164	0.0379
222A	November 11, 1924	0.677	0.0961	0.0861	None	0.0125	0.0118	0.0122	0.0059	0.0433
						As percentages of moisture free weight of material				
138	May 13, 1924	3.610	0.3000	0.2500	0.0085	0.0402	0.0121	0.0520	0.0350	0.0950
140	May 22, 1924	3.415	0.3400	0.2790	0.0065	0.0615	0.0180	0.0700	0.0330	0.0920
166	June 11, 1924	2.210	0.2440	0.2200	0.0040	0.0465	0.0092	0.0550	0.0310	0.0730
189	July 16, 1924	2.150	0.2830	0.2550	0.0038	0.0545	0.0070	0.0600	0.0320	0.0990
195	August 28, 1924	2.080	0.2580	0.2240	0.0040	0.0350	0.0194	0.0400	0.0280	0.0980
210	October 7, 1924	1.845	0.2310	0.2060	None	0.0350	0.0200	0.0420	0.0390	0.0900
222A	November 11, 1924	1.550	0.2200	0.1970	None	0.0285	0.0270	0.0280	0.0135	0.0990

TABLE III (Concluded)

TOTAL NITROGEN AND TOTAL WATER-SOLUBLE NITROGEN IN THE NEW (1924) BRANCH GROWTH, THE ONE YEAR (1923) BRANCH GROWTH AND LEAVES, TOGETHER WITH THE DISTRIBUTION OF THE NON-PROTEIN CONSTITUENTS EXPRESSED AS PERCENTAGES OF FRESH AND MOISTURE FREE WEIGHTS, RESPECTIVELY
LEAVES (TREE NO. E-20)

SERIES NO.	COLLECTING DATE	TOTAL N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	AMMONIA N	AMINO N	AMIDE N	BASIC N	HUMIN N	RESIDUAL N
			As percentages of fresh weight of material							
106	May 13, 1924.....	0.860	0.0637	0.0526	0.0015	0.0076	0.0028	0.0122	0.0097	0.0188
129	May 22, 1924.....	0.782	0.0584	0.0478	0.0012	0.0100	0.0043	0.0150	0.0078	0.0097
153A	June 11, 1924.....	0.681	0.0578	0.0530	0.0019	0.0096	0.0028	0.0148	0.0103	0.0135
180	July 16, 1924.....	0.663	0.0612	0.0563	0.0014	0.0107	0.0014	0.0213	0.0096	0.0119
199	August 28, 1924.....	0.672	0.0571	0.0510	None	0.0097	0.0039	0.0135	0.0112	0.0127
215	October 7, 1924.....	0.543	0.0539	0.0480	None	0.0090	0.0052	0.0139	0.0099	0.0080
233A	November 11, 1924 ..	0.443	0.0493	0.0437	None	0.0068	0.0071	0.0082	0.0107	0.0190
			As percentages of moisture free weight of material							
106	May 13, 1924.....	3.520	0.2300	0.1900	0.0055	0.0275	0.0101	0.0440	0.0350	0.0677
129	May 22, 1924.....	3.400	0.2540	0.2080	0.0050	0.0435	0.0185	0.0650	0.0340	0.0420
153A	June 11, 1924.....	1.980	0.1680	0.1540	0.0055	0.0280	0.0082	0.0430	0.0300	0.0393
180	July 16, 1924.....	1.926	0.1780	0.1637	0.0040	0.0312	0.0040	0.0620	0.0280	0.0345
199	August 28, 1924.....	1.740	0.1480	0.1320	None	0.0250	0.0100	0.0350	0.0290	0.0330
215	October 7, 1924.....	1.560	0.1550	0.1320	None	0.0260	0.0150	0.0400	0.0285	0.0230
233A	November 11, 1924 ..	1.240	0.1380	0.1225	None	0.0190	0.0200	0.0230	0.0300	0.0305

ACCURACY AND LIMITS OF ERROR

Table IV gives the differences between duplicate determinations in certain of the series calculated as percentages of the mean readings.

TABLE IV
DIFFERENCES OBTAINED IN CERTAIN DUPLICATE DETERMINATIONS

SERIES NO.	TOTAL N	TOTAL WATER-SOLUBLE N	α -MONO-AMINO N	AMIDE N
	Per cent.	Per cent.	Per cent.	Per cent.
60	0.48	1.60	6.75	7.80
76	1.20	7.20	3.00
114	0.40	1.30	4.60	7.10
129	0.14	5.80	1.00
138	0.30	1.20	3.90	4.50

The total nitrogen shows a maximum difference of 0.48 per cent.; the water-soluble nitrogen of 1.60 per cent.; the α -mono-amino nitrogen of 7.20 per cent.; and the amide nitrogen of 7.80 per cent. All variations in the metabolism figures, therefore, can be regarded as significant.

Discussion and conclusions

TOTAL NITROGEN

TOTAL NITROGEN IN THE ONE YEAR BRANCH GROWTH.—It was pointed out in the third paper (7) that during the first growth of spring translocation of nitrogen from the one and two year branches to the young shoots is very marked. The effect of this demand is strikingly illustrated in the present investigation, as shown in fig. 1. The total nitrogen of the one year growth of the untreated tree drops during the period from April 28 to May 13 from 0.288 per cent. to 0.166 per cent. on the fresh weight basis, *i.e.*, from 0.720 per cent. to 0.480 per cent. on the moisture free basis. On May 13 the leaves contain as high as 0.860 per cent. nitrogen on the fresh weight basis and 3.520 per cent. on the moisture free basis.

The effect of the application of 10 pounds of NaNO_3 on April 20 to tree no. E-20 is distinctly evident in both the quantitative and qualitative relationships. After May 13, when the trees are in full bloom, the total nitrogen curves of the two trees take a different course. Basing observations on the moisture free basis (dy/dt), *i.e.*, the slope of the total nitrogen curves of the untreated tree is negative to the end of September; whereas in the treated tree (dy/dt), though fluctuating considerably, shows a net change of almost zero. In other words, from May 13 to the end of September there has been

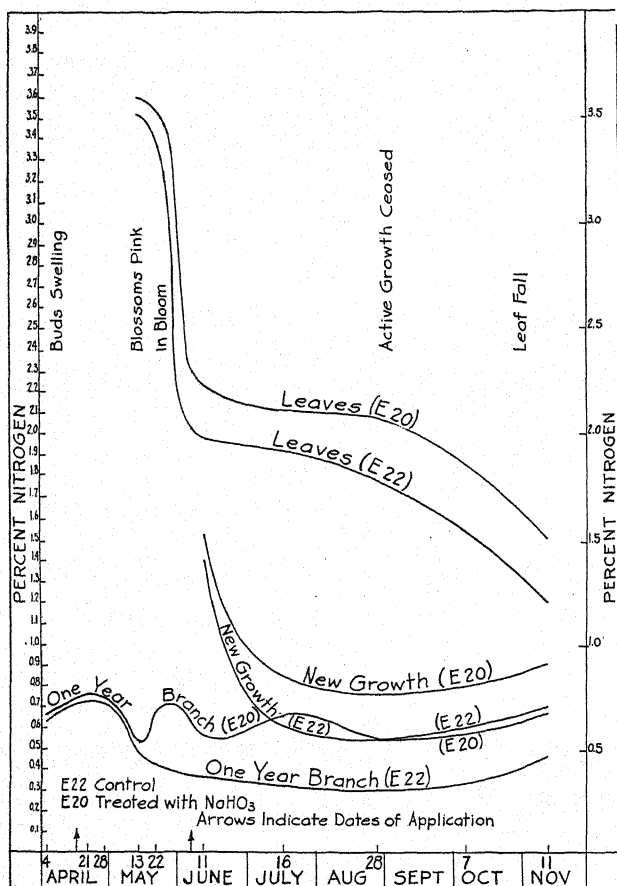


FIG. 1. Total nitrogen in the leaves, new (1924) branch growth and one year (1923) branch growth.

no net gain or loss in total nitrogen in the one year (1923) branch growth of the treated tree; whereas in the unfertilized tree the total nitrogen of the one year (1923) branch growth decreased 35 per cent. during the same period.

The increase in the total nitrogen content of the one year branch growth of tree no. E-20 continues until it is utilized by the demands of the developing shoots. An inspection of the curves shows that the second application (June 8) of 10 pounds of NaNO_3 to E-20 is absorbed more rapidly than the first application. These facts lend further support to the suggestion that the rate of absorption of a nutritive element is fundamental and probably one of the most important factors in soil fertility and plant nutrition problems.

The differences in total nitrogen content as a result of fertilization are again clearly indicated at the period when the trees enter the rest period. On April 4 the one year branch growth of the untreated tree has 0.299 per cent. nitrogen, but on November 11 only 0.208 per cent. on the fresh weight basis, *i.e.*, 0.635 per cent. and 0.437 per cent., respectively, on the moisture free basis; whereas the treated tree has 0.263 per cent. and 0.346 per cent., *i.e.*, 0.628 per cent. and 0.690 per cent. on the fresh and moisture free basis, respectively, on the respective dates. The results are clearly shown in table V.

TABLE V

COMPARISON OF THE PERCENTAGES OF TOTAL NITROGEN OF THE ONE YEAR BRANCH GROWTHS OF EACH TREE AT CERTAIN PERIODS

COLLECTING DATE	UNTREATED		TREATED	
	Fresh weight basis	Moisture free basis	Fresh weight basis	Moisture free basis
April 4, 1924.....	0.299	0.635	0.263	0.628
April 28, 1924.....	0.288	0.720	0.329	0.764
November 11, 1924.....	0.208	0.437	0.346	0.690

TOTAL NITROGEN OF THE NEW (1924) BRANCH GROWTH.—The differences in total nitrogen content of the new (1924) branch growth are also quite marked on the two trees. The untreated tree had a total nitrogen content of 0.379 per cent. on June 11 and of 0.300 per cent. on November 11 on the fresh weight basis, *i.e.*, 1.40 per cent. and 0.603 per cent., respectively, on the moisture free basis; whereas the treated tree had a total nitrogen content of 0.432 per cent. and 0.517 per cent. on the fresh weight basis, *i.e.*, 1.503 per cent. and 0.900 per cent., respectively, on the moisture free basis on these dates.

Table VI conveniently shows the effect of the NaNO_3 applications on the total nitrogen content of the new growth.

It has already been shown in the third paper that the results of applying 5 pounds of nitrogen are only just about sufficient to maintain nitrogen equilibrium under the conditions of that experiment. Considering, then, only the season's (1924) branch growth and the one year (1923) branch growth, the present results show that whereas the untreated tree will start the next season on a much lower nitrogen plane than in the previous season, the treated tree has slightly more than maintained equilibrium. This appears to be in accordance with the practical results obtained in this Experiment Station, indicating that 10-pound applications of NaNO_3 per tree may be practicable economically.

TABLE VI

COMPARISON OF THE PERCENTAGES OF TOTAL NITROGEN OF THE NEW (1924) BRANCH GROWTHS OF EACH TREE AT CERTAIN PERIODS

COLLECTING DATE	UNTREATED		TREATED	
	Fresh weight basis	Moisture free basis	Fresh weight basis	Moisture free basis
June 11, 1924.....	0.379	1.400	0.432	1.503
November 11, 1924.....	0.300	0.604	0.517	0.900

TOTAL NITROGEN IN THE LEAVES.—Table VII shows the effect of fertilization on the total nitrogen content of the leaves. The effect on the leaves is more clearly indicated in the diminution in concentration of nitrogen as indicated by the much lower figures for the untreated tree on the fresh weight basis than in the absolute quantities.

TABLE VII

COMPARISON OF THE PERCENTAGES OF TOTAL NITROGEN OF THE LEAVES OF EACH TREE AT CERTAIN PERIODS

COLLECTING DATE	UNTREATED		TREATED	
	Fresh weight basis	Moisture free basis	Fresh weight basis	Moisture free basis
May 13, 1924.....	0.860	3.520	0.830	3.610
November 11, 1924.....	0.443	1.240	0.677	1.550

Although the character of the curves is the same, there are decided quantitative differences. On May 13 the total nitrogen content of the young leaves of the untreated tree is 0.860 per cent. and of the treated tree 0.830 per cent. (fresh weight basis), or 3.520 per cent. and 3.610 per cent. (moisture free basis), respectively. At the end of the period of chlorophyll degeneration, about the time of leaf fall, the untreated tree contained 0.443 per cent. and the treated tree 0.677 per cent. (fresh weight basis), or 1.240 per cent. and 1.550 per cent. (moisture free basis), respectively.

The same fall in total nitrogen content of the leaves from the time of bud opening to the middle of June, observed in the previous investigation and described in the third paper of the series, takes place in both trees. This corresponds to the period of most rapid growth.

THE NITROGEN DISTRIBUTION

THE PARTITION OF NITROGEN IN THE LEAVES.—The marked differences in the nitrogen metabolism of the two trees is very evident from an inspection

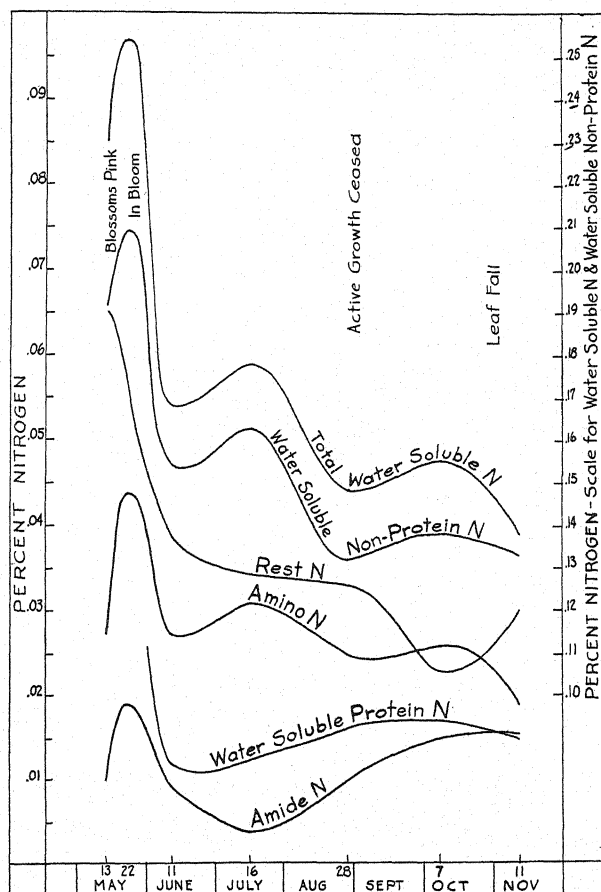


FIG. 2. Total water-soluble N, water-soluble protein N, non-protein N, amino N, amide N and "rest" N in the leaves of the untreated tree (no. E-22).

of figs. 2 and 3. The analytical results are given in table III. The most striking differences are in the total water-soluble nitrogen, the non-protein nitrogen, the amino nitrogen, and the "rest" nitrogen. The effect of the second application of NaNO_3 on June 8 results in a large increase of total water-soluble and non-protein nitrogen. Thus, in the treated tree the increase in concentration (as indicated by the fresh weight results) of water-soluble N and non-protein N from June 11 to July 16 amounts to 43 and 16 per cent., respectively, but during the same period the total water-soluble and non-protein nitrogen of the leaves of the untreated tree show an increase of only 6 and 8 per cent., respectively. The earlier investigations show a similar fluctuation, occurring, however, a month later. That the increase at

this period in the untreated tree can be due solely to increased nitrification of the soil is quite clear, for fig. 4 shows that the one year (1923) branch growth increases in total water-soluble nitrogen also.

The course followed by the amino and amide nitrogen in the leaves calls for no special comment. The curves are similar to those described in the earlier investigations (7). It is to be noted that they are much higher in the fertilized tree as a result of the NaNO_3 treatment.

The "rest" nitrogen compounds represent, both in absolute magnitude and in their metabolic gyrations, an extremely important group in that the evidence indicates that these unclassified compounds form an important link in the degradation of proteins (7). The fluctuations of this fraction in the two trees show more remarkable differences than any of the other fractions.

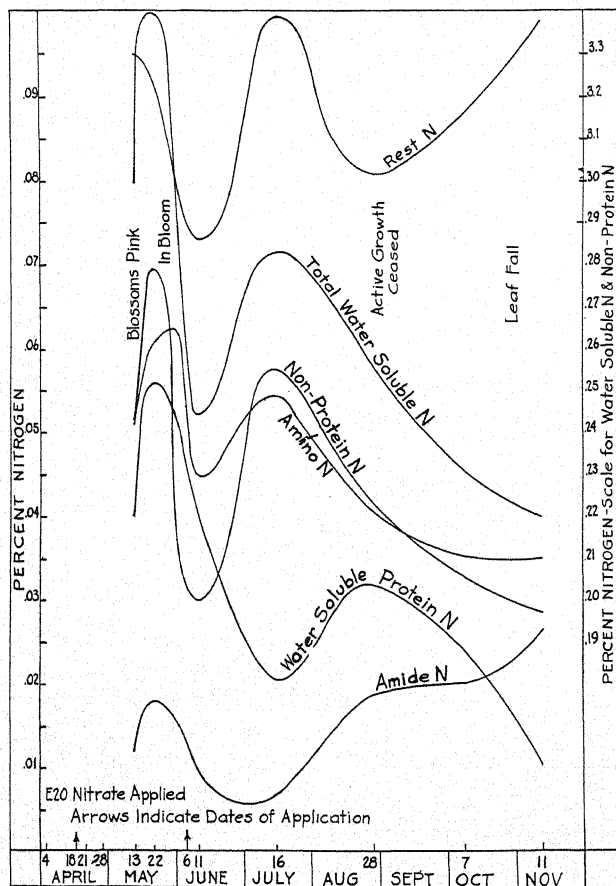


FIG. 3. Total water-soluble N, water-soluble protein N, non-protein N, amino N, amide N and "rest" N in the leaves of the treated tree (no. E-20).

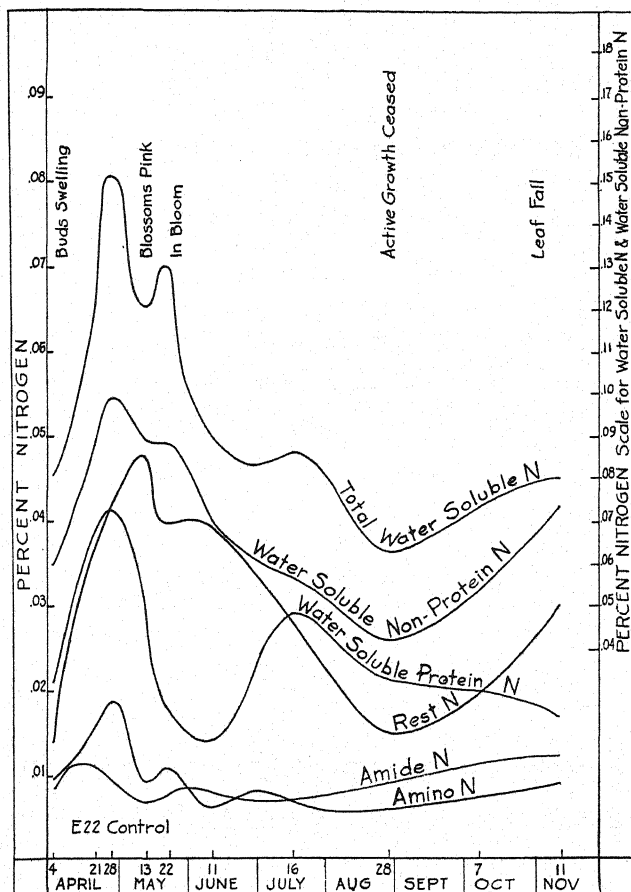


FIG. 4. Total water-soluble N, water-soluble protein N, non-protein N, amino N, amide N and "rest" N in the one year (1923) branch growth of the untreated tree (no. E-22).

The positive slope of the "rest" nitrogen curve in fig. 3 just after the application of nitrate to the treated tree on June 8 is a characteristic of this tree only; the leaves of the untreated tree do not show it. It is hoped to be able to isolate and identify some of these unclassified nitrogen compounds later.

THE PARTITION OF NITROGEN IN THE ONE YEAR (1923) BRANCH GROWTH.—The conclusions drawn in the earlier investigations are, in general, confirmed in this investigation; and it will, therefore, be unnecessary to discuss them in detail again. The visual presentation by means of figs. 4 and 5 shows very clearly the differences between the treated and untreated trees.

The influence of the sodium nitrate addition on the nitrogen partition on the one year (1923) branch growth of tree no. E-20 on April 20 and

June 8 is very marked. From a comparison of the cycle curves (figs. 4 and 5) it is readily seen that the period of absorption is characterized by a marked increase in the total water-soluble and the non-protein nitrogen with concomitant increases in the amino, amide and basic nitrogen of tree E-20 (treated) in comparison with tree E-22 (untreated).

On April 21 the percentages of total water-soluble nitrogen in the one year (1923) branch growth of trees E-20 (treated) and E-22 (untreated) are 0.0463 and 0.0520 per cent., respectively, but three weeks later the quantities present are 0.0789 and 0.0385 per cent., respectively—an increase of 85 per cent. and a decrease of 30 per cent., respectively. Again, at the last sampling, November 11, tree no. E-20 (treated) has 0.0852 per cent.,

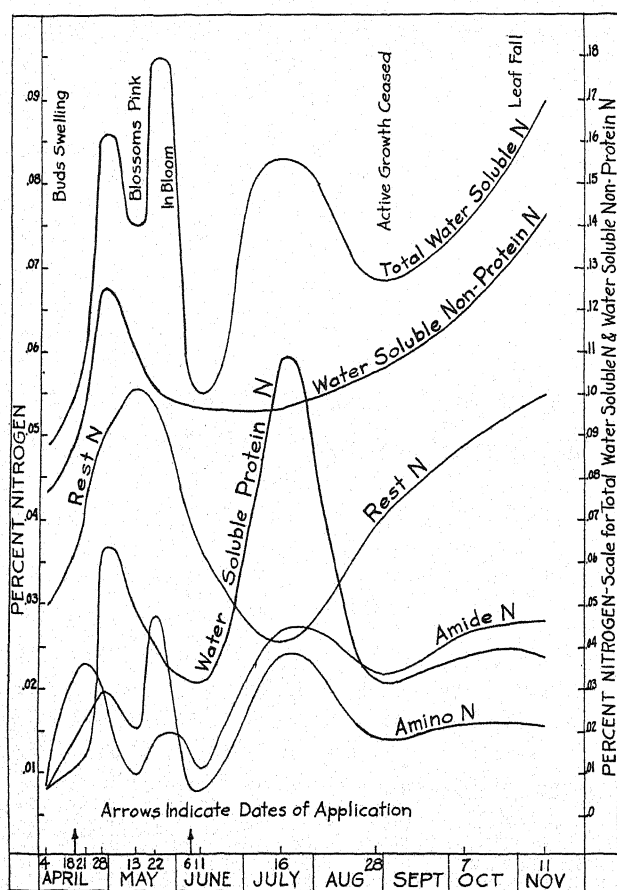


FIG. 5. Total water-soluble N, water-soluble protein N, non-protein N, amino N, amide N and "rest" N in the one year (1923) branch growth of the treated tree (no. E-20).

but tree no. E-22 (untreated) has only 0.0432 per cent. of water-soluble nitrogen. The non-protein nitrogen results of the trees parallel these figures, *viz.*, 0.0380 and 0.0389 per cent. on April 21 and 0.0429 and 0.0375 per cent. three weeks later.

The mono-amino and amide nitrogen curves, too, are interesting. Up to May 10 (about three weeks after the first application of NaNO_3 to E-20) the curves for amino N show little differences in the two trees. About this date, however, the mono-amino and amide nitrogen begin to increase rapidly in the treated tree. The same phenomena are observed after the second application of NaNO_3 on June 8. As already pointed out, at this time of the year absorption of the NaNO_3 and its transformation to $-\text{NH}_2$ and $-\text{CO.NH}_2$ groups is more rapid, for the effect is noticeable in the leaves, one year branch growth and new growth within ten days of the second application.

The importance of the "rest" nitrogen compounds both in magnitude and in their metabolic gyrations is again clearly indicated in the one year (1923) branch growth. Their significance is more or less a mystery, but an inspection of the graphs will readily confirm the conclusions previously drawn (7) where it was suggested that they must take part in the degradation of proteins.

THE PARTITION OF NITROGEN IN THE NEW (1924) BRANCH GROWTH.—The differences in the nitrogen partition in the new (1924) branch growth are more marked than in the one year (1923) branch growth. The behavior of the treated tree (E-20) with respect to the total water-soluble and non-protein nitrogen cycle corresponds to the results obtained in the earlier investigations (7). The main point of interest is the steep positive slope as shown in fig. 7, about a week after the second application of NaNO_3 (June 8), and the very large increase again in these constituents during the fall storage of nitrogen.

A complete interpretation of the total water-soluble and non-protein nitrogen cycles (fig. 6) of the untreated tree is more difficult. It is apparent that the concentrations of nitrogen in both fractions are much lower in the check tree than in the nitrogen treated tree and that these differences are associated with differences in vigor.

The mono-amino acid and amide nitrogen cycles in the new (1924) branch growth of the two trees show the characteristic differences already observed in the one year (1923) branch growth, *e.g.*, the effect of the second application of NaNO_3 on June 8 to tree E-20 is very evident from the marked increase in amino N and the "rest" N compounds. The large storage as amide N in the new branch growth as a result of fertilization is very marked; thus, on November 11 the amide nitrogen in E-20 is 0.0201 per cent. and 0.0350 per cent., but in E-22 only 0.0087 per cent. and 0.0180 per

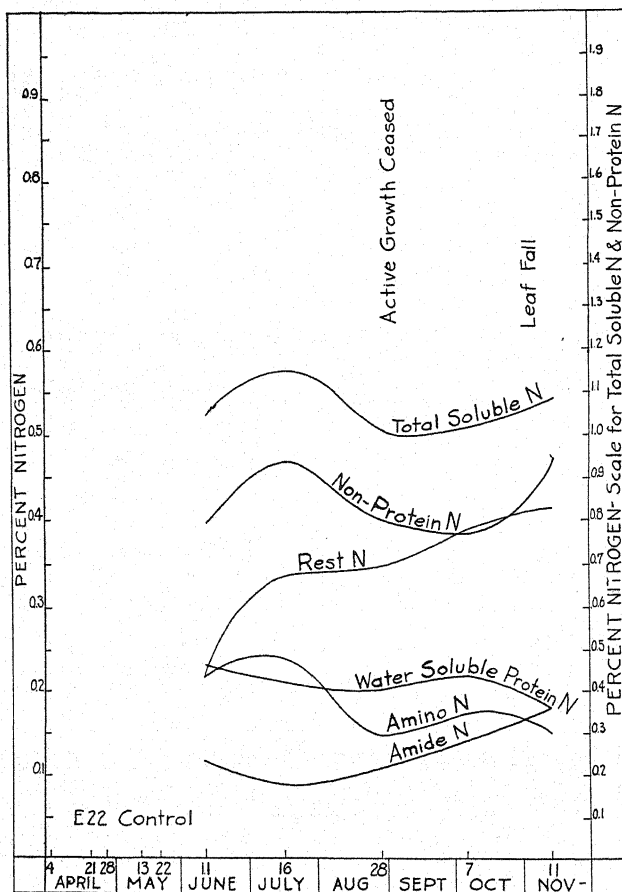


FIG. 6. Total water-soluble N, water-soluble protein N, non-protein N, amino N, amide N and "rest" N in the new (1924) branch growth of the untreated tree (no. E-22).

cent. on the fresh and moisture free basis, respectively, and the mono-amino N 0.0281 per cent. and 0.0490 per cent. and 0.0073 per cent. and 0.0150 per cent. on the fresh and moisture free basis, respectively.

Summary and conclusions

These studies are an extension of the partition investigations reported in the third paper of the series (7) to two Stayman Winesap trees of the same age, growing on sod in a homogenous soil. One of these trees was fertilized with NaNO_3 and the other left unfertilized. In general, the present findings confirm the conclusions drawn from the previous studies.

The present investigations have shown that:

1. The total nitrogen of the leaves and shoot growth vary with growth. During the early growth of spring the demand of the young shoots upon the nitrogen of the one year (1923) branch growth is very marked. This is especially shown in the untreated tree, E-22.
2. The effect of the two 10-pound applications of sodium nitrate on April 20, 1924, and June 8, 1924, on the total nitrogen content of the two trees may be thus summarized:
 - (a) The total nitrogen content of the one year (1923) branch growth of the fertilized tree from the period of full bloom to the end of September increased 10 per cent., but the total nitrogen of the

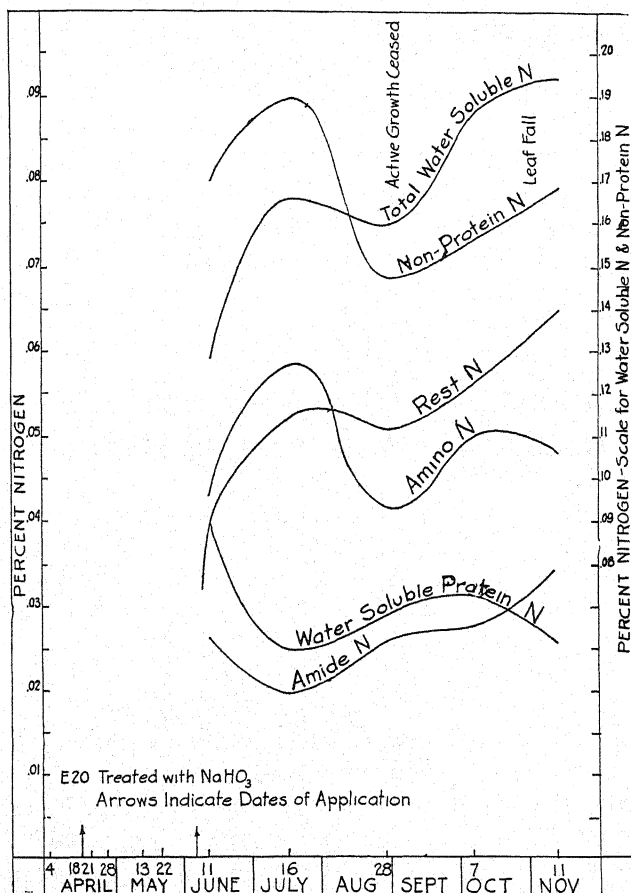


FIG. 7. Total water-soluble N, water-soluble protein N, non-protein N, amino N, amide N and "rest" N in the new (1924) branch growth of the treated tree (no. E-20).

one year (1923) branch growth of the unfertilized tree decreased 35 per cent. during this period.

(b) From June 11 to November 11 the total nitrogen of the new growth of the treated tree (E-20) decreased 40 per cent., but that of the untreated tree (E-22) decreased 57 per cent.

(c) The leaves of the untreated tree are consistently lower than in the treated tree.

A much lower plane of nitrogen metabolism is thus indicated in the unfertilized tree.

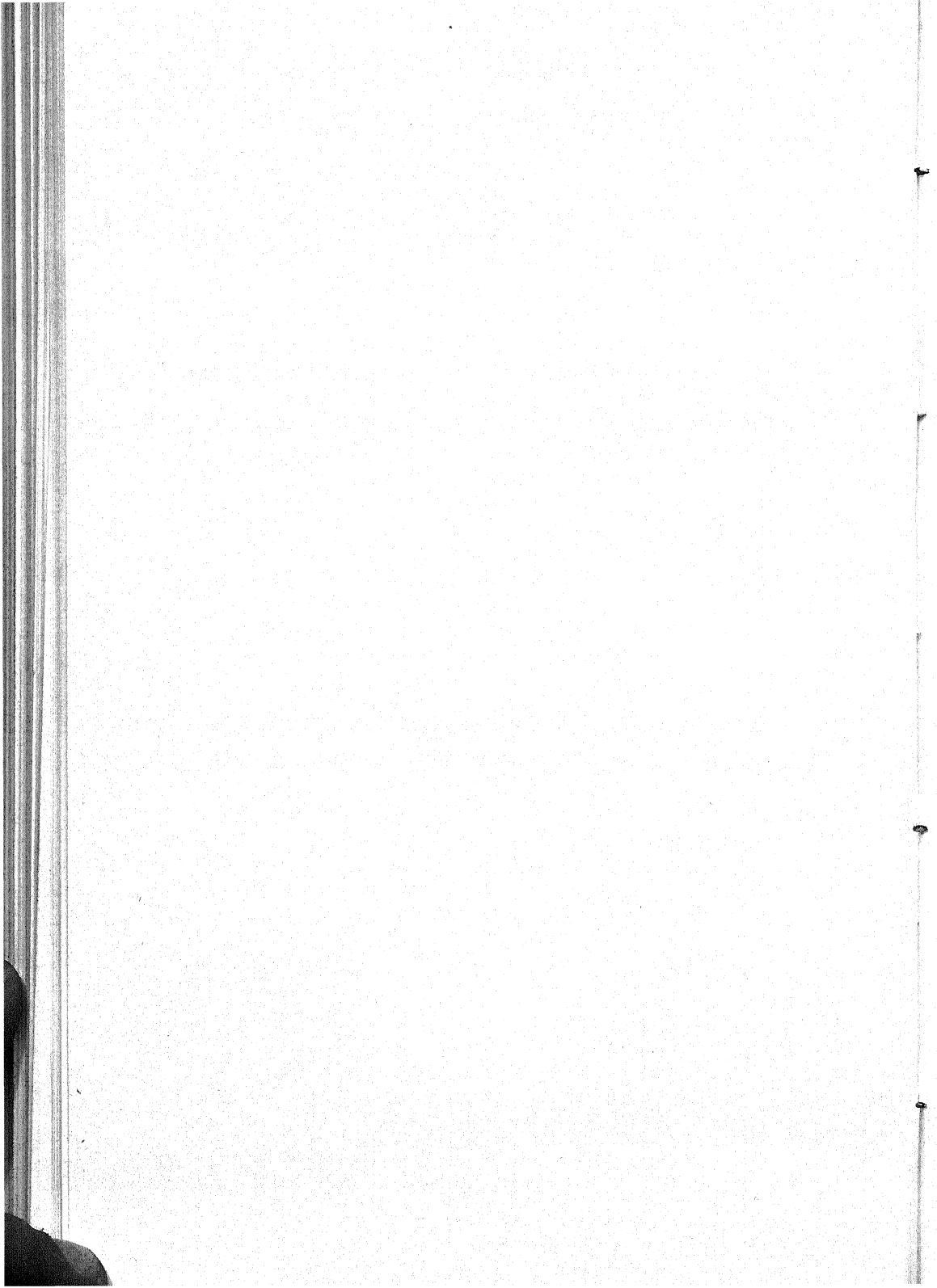
3. The period of most rapid absorption of the NO_3 ion is characterized by a large increase in the total water-soluble and non-protein nitrogen, concomitant with which there is an increase in the amino, amide, basic, and "rest" nitrogen fractions of the fertilized as compared with the unfertilized tree in the leaves, the one year (1923) branch growth and the new (1924) branch growth.
4. From the course of the curves for total water-soluble and non-protein nitrogen or amino N of the fertilized as compared with the unfertilized tree, it can be deduced that the first application of sodium nitrate on April 20 took three weeks to reach the one year branches. The same curves show that the absorption and translocation of the NO_3 ions of the second application on June 6 is much more rapid, taking about one week only to get to the more metabolically active parts of the tree.
5. The total water-soluble nitrogen and non-protein nitrogen is decidedly higher in the metabolically active parts of the treated tree as compared with the untreated tree throughout the whole cycle. These differences correspond with the relative vigor of the trees and must be associated with differences in enzymatic activity. Taken in conjunction with the close parallelism of the amino-acid fraction with the total water-soluble and non-protein fractions (7), these findings suggest a possible simplification of the nitrogen distribution problem in practical horticulture.
6. Terminal growth was decidedly greater in the fertilized than in the unfertilized tree.
7. Nitrogen is stored in the fall as amide N and as "rest" N compounds. The storage of these compounds is much higher in the treated tree than in the untreated tree. This fact is of extreme importance in connection with the demands of the young shoots in the spring. This is true of both the one year (1923) branch growth and the new (1924) branch growth.
8. The "rest" nitrogen compounds form an important connecting link in the synthesis and degradation of proteins.

The writer desires to thank Mr. C. A. KERN for the determinations of the hygroscopic and imbibitional water and Miss ETHEL GINGRICH for assistance in calculating and checking the computations.

DEPARTMENT OF AGRICULTURAL AND BIOLOGICAL CHEMISTRY,
AGRICULTURAL EXPERIMENT STATION,
THE PENNSYLVANIA STATE COLLEGE.

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RELATION OF COMPOSITION TO GROWTH AND FRUITFULNESS OF YOUNG APPLE TREES AS AFFECTED BY GIRDLING, SHADING, AND PHOTOPERIOD¹

R. H. ROBERTS

(WITH TWENTY-TWO FIGURES)

Introduction

An effort is being made to record the relations of internal composition of apple trees to their vegetative responses, especially to the particular growth characters accompanying blossom bud formation. Various environmental conditions are being used to vary the plant composition. In a previous report (4) the effects of nitrogen fertilization were considered. It was observed that the vegetative condition as measured by such characters as length of growth, leaf colors, bark colors, and plant composition varied directly with the nitrogen fertilization under the cultural conditions provided: dwarf trees in pots in the greenhouse. On the other hand, blossom bud formation was not correlated to cultural treatment, but rather to growth character and to a "balance" in composition when the carbohydrate and nitrogen content are compared. This is a condition similar to that described by KRAUS and KRAYBILL for fruit development in the tomato (2). The most vegetative plants or growths were highest in nitrogen, lowest in carbohydrates, and unfruitful; the least vegetative plants were lowest in nitrogen, highest in carbohydrates and were unfruitful; and the moderately vegetative plants were intermediate in nitrogen and carbohydrate content and were fruitful.

The significance of the above relation of composition to blossom bud formation and fruiting lies in this: if reproduction is related to growth character, which is the response to internal composition, rather than directly to cultural treatment, then, (1) we are provided with a basis for interpreting the conflict of results secured from the limiting factor or plot method of investigating tree performance, and (2) we can use the growth character of the tree as the basis for diagnosing orchard conditions when attempting to decide upon needed cultural treatments. Instead of using the present unsuccessful method of trying to duplicate a cultural treatment which is reported as being profitable in another locality, the practical grower

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can attempt to produce by any feasible means and with a reasonable assurance of success, such amounts and types of growth as give the desired fruiting responses. To illustrate, the use of readily available nitrogen fertilizers has become very general in recent years, because of the frequent increase in fruiting which results. In situations where low production is due to an over-vegetative type of growth, nitrogen fertilization tends further to delay or reduce production. A similar condition pertains as regards pruning and cultivation; these may be harmful as well as beneficial to fruiting, depending upon the vegetative condition of the trees being considered.

Materials and methods

The data presented herein were collected to determine the effect of three other environmental conditions upon tree composition, blossom bud formation, and to further test whether or not fruitfulness was related directly to the treatment or to the balance in composition produced by the treatments. The conditions used were girdling, shading, and reduced photoperiod.

As regards shading and girdling, one hundred yearling Wealthy trees grafted on standard seedling stocks were planted in early May, 1926. When the new growth averaged one to two inches long, one half of the row was covered with a burlap shade on a lath frame. Further, one half of the shaded and of the unshaded trees were girdled by removing a half inch of bark about mid-height. The girdle was covered with grafting wax. Several girdled trees died. Many girdled-and-shaded trees died. The trouble was obviously due to lack of root development. No trees were lost from girdling in late July after appreciable root development had occurred. Some sucker growth was produced below the girdles. A few trees produced sufficient callus to bridge the girdle late in the growing season. These latter were avoided when sampling for chemical analyses. The effect of shading is probably less than would ordinarily obtain, as the sunshine from June to November was only 41.5 per cent. of possible or 69.4 per cent. of normal in 1926.

Results

The growth produced under the different treatments is shown in table I. The growth data were obtained from averages of paired trees. That is, the "check" is made up of trees having a previous season length and weight like the treated trees. Thus each treatment has a separate check. There is considerable difference in checks because the girdled trees which died were smaller than the ones which lived and because the larger check trees tended to grow more than the smaller ones.

There are some items of especial interest in table I:

1. The greatest amount or quantity of growth when measured by per cent. gain in weight of tree, total length of shoots, or amount of new roots was made

TABLE I
EFFECT OF SHADE AND GIRDLING UPON GROWTH OF YOUNG APPLE TREES

TREATMENT	IN- CREASE IN WEIGHT	LENGTH TERMINAL BRANCH	DIAMETER TERMINAL GROWTH	INTER- NODE LENGTH	TOTAL SHOOTS		RATIO TOTAL SHOOTS TO PER CENT. WEIGHT IN- CREASE	GREEN WEIGHT OF NEW ROOTS ²
					NUM- BER	LENGTH		
	Per cent.	cm.	mm.	mm.		cm.		gm.
Sun, un- girdled	162.9	37.2	2.86	24.5	3.35	97.0	0.60	7.43
Shade, un- girdled	51.4	40.4	2.51	30.0	2.93	85.2	1.66	2.67
check	135.6	32.6	2.69	23.6	2.93	71.3	0.53	
Sun, girdled	40.6	12.1	3.40	20.7	3.66	52.2	1.29	2.48
check	154.5	40.9	2.68	23.1	3.00	86.3	0.56	
Shade, girdled	19.6	21.2	3.08	27.4	3.20	49.2 ¹	2.51	0.49
check	168.5	36.7	3.02	25.8	3.40	104.3	0.62	

¹ Less branching as well as less growth of suckers than sun and girdling.

² Average of three trees.

by the ungirdled sun trees. The least was made by the girdled, shaded trees.

2. On the other hand, the extremes of kind or quality of growth when measured by such characters as individual shoot length, diameter and internode distance are found in the shaded trees and the girdled trees in the light.
3. The differences in character of growth above and below the girdles, as will be pointed out later, make an error in measuring total growth of tree. For instance, the seasonal increase in weight of shaded and girdled trees (average 10.8 gm.) is less in some cases than the increase above the girdle, thus showing a loss of weight below the girdle.

The failure to differentiate between the amount and character or quality of growth is believed to be a frequent error in interpreting tree growth data secured from cultural plots.

Further details of growth character as affected by the shading and girdling are presented in tables II and III and figs. 1 to 19.

Some responses of particular interest, most of which are recorded in the tables or illustrations follow:

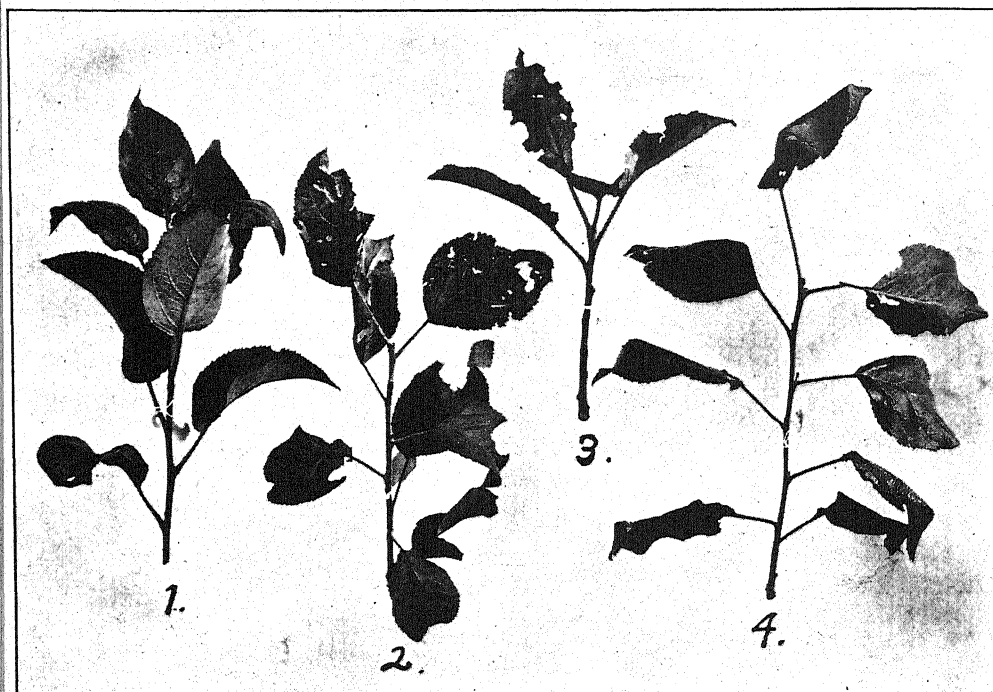


FIG. 1. Character of shoot and foliage growth: Left to right, 1. Sun; 2. Shade; 3. Sun and girdled; 4. Shade and girdled. The leaves of 1 and 4 are very similar in texture although appearing different due to a difference in orientation.

TABLE II

EFFECT OF SHADE AND GIRDLING UPON FOLIAGE DEVELOPMENT OF YOUNG APPLE TREES*

TREATMENT	LEAF SIZE	LEAF THICKNESS		CELLS PER SQ. MM.		STOMATA PER SQ. MM.	MILLION CELLS PER LEAF SURFACE	
		ABOVE GIRDLE	BELOW GIRDLE	UPPER SURFACE	LOWER SURFACE		LOWER	UPPER
	sq. in.	mm.	mm.					
Sun	4.08	0.276	0.272	1737	1920	583	4.57	5.06
Shade	6.12	0.153	0.161	1385	1895	460	5.46	7.46
Sun, girdled ..	2.18	0.289	0.255	2158	2772	820	3.03	3.90
Shade, girdled	5.00	0.158	0.145	1961	2468	580	6.32	7.72

* Details of leaf and stem structure recorded by MARIAN DEATS ABEGG.

TREATMENT	NEW GROWTH										NEW XYLEM		
	RADIUS			RATIO			NUMBER CELLS IN RADII				TRUNK		ROOTS
	PITH	XYLEM	BARK*	$\frac{\text{PITH}}{\text{XYLEM}}$	$\frac{\text{PITH}}{\text{BARK}}$	$\frac{\text{XYLEM}}{\text{BARK}}$	PHLOEM	RAYS	XYLEM	UPPER	LOWER		
Sun	mm. 0.622	mm. 0.306	mm. 0.610	2.03	1.02	0.50	15	8	18	mm. 0.68	mm. 0.74	mm. 0.63	
Shade	0.585	0.263	0.479	2.22	1.22	0.55	11	7	13	0.23	0.38	0.24	
Sun, girdled	0.620	0.502	0.722	1.23	0.86	0.70	18	18	39	0.81	0.34	0.16	
Shade, girdled...	0.577	0.381	0.565	1.51	1.02	0.67	13	9	20	0.30	0.07	0.04	

* Phloem and cortex.

1. The influence of the treatments upon diameter growth.
2. The marked reduction in secondary thickening in the shade and below girdles. Bast fibre development was also nearly absent. Only the check trees had second year bast in the lower trunks.
3. Ray cell shapes varied greatly as well as vessel, wood-fibre, and wood-parenchyma cell sizes.
4. Starch grain sizes and storage cell wall thickenings were markedly affected.
5. Leaf palisade was strikingly reduced by shade.
6. Large leaves on shade trees resulted from both larger cells and greater number per leaf.
7. Girdling and shading greatly reduced root development. This is correlated with the effect upon new xylem production.

Notes:

1. Three trees were used for each sample.
2. A modified Kjeldahl was not used in determining total nitrogen, as apple wood is rarely found to contain an appreciable amount of nitrates.
3. Ptyalin digestion was used in extracting starch.
4. The reducing substances were obtained by the Munson-Walker method and were measured by the Shaffer-Hartman titration method. The volumetric method gives results with mature apple wood comparable to the gravimetric determinations.
5. Acid hydrolysis was accomplished by refluxing with 2.5 per cent. sulphuric acid for one hour.
6. The bark was not analyzed separate from the wood. This may be a source of error. In general, however, it seems that bark and wood analyses vary more in percentages, due obviously to a greater inert fraction in the wood, than in the direction of difference. While they may show decided differences in percentage the fluctuations in amounts are parallel.

Table IV shows the usual lack of relation between the starch fraction and either the reducing or acid hydrolyzable fractions. As usual, these latter are not correlated with vegetative condition or anatomical structure.

The relatively high starch content below the girdles in unshaded trees is obviously due to slight utilization of reserves, the small top growth made before the time of girdling being from material near the growing points and from newly synthesized foods.

The intermediate carbohydrate content and nitrogen carbohydrate relation of new shoots on sun and shade-and-girdled trees as compared to the shaded and sun-and-girdled trees is in agreement with their intermediate growth character.

The low nitrogen content of the wood and roots below the girdles, especially in the shade, was a surprising condition. The growth character of the suckers arising from this region were of a characteristic "high nitrogen" appearance. As there is a greater reduction in carbohydrate percentage than in nitrogen percentage below the girdles, the lower portion of the stems do have a "high nitrogen" condition. This is taken as evidence that growth

TABLE IV
EFFECT OF SHADE AND GIRDLING UPON COMPOSITION OF YOUNG APPLE TREES

SAMPLE	TREATMENT	MOISTURE	SPECIFIC GRAVITY	TOTAL N	STARCH	REDUCING SUBSTANCE		ACID HYDROLYZABLE	TOTAL CARBOHYDRATE	RATIO TO N	
						FREE	TOTAL			STARCH	TOTAL CARBOHYDRATE
One year wood	Sun	Per cent. 52.1	1.115	Per cent. 0.876	Per cent. 7.40	Per cent. 3.84	Per cent. 6.53	Per cent. 6.05	Per cent. 19.98	8.45	23.12
	Shade	54.9	1.084	0.869	6.61	3.37	7.76	3.73	18.10	7.61	21.08
	Sun, girdled	50.2	1.356	0.775	9.92	4.13	8.28	7.65	25.85	13.15	33.40
Two year wood, upper half	Shade, girdled	51.3	1.207	0.953	8.88	3.50	7.48	4.31	20.67	9.32	21.70
	Sun	46.3	1.106	0.557	5.82	3.57	5.14	4.08	15.04	10.46	27.10
	Shade	48.1	1.008	0.668	5.07	3.46	4.92	4.66	14.65	7.58	21.95
Two year wood, lower half	Sun, girdled	46.6	1.056	0.540	8.29	3.89	6.28	6.48	21.05	15.36	38.99
	Shade, girdled	48.3	1.009	0.582	7.40	5.52	7.75	5.48	20.63	12.72	35.50
	Sun	44.7	1.005	0.528	4.97	1.81	3.13	2.99	11.09	9.40	21.05
Two year roots	Shade	47.1	1.042	0.526	4.47	2.26	3.23	4.38	12.08	8.49	23.00
	Sun, girdled	48.6	1.010	0.443	4.57	1.80	3.50	4.04	12.11	10.32	27.35
	Shade, girdled	52.6	0.961	0.376	4.92	1.47	2.97	2.96	6.85	2.45	18.23
Two year roots	Sun	55.2	1.049	0.813	14.20	4.13	6.11	4.29	24.60	17.50	30.30
	Shade	56.8	1.002	0.963	12.42	4.16	6.32	5.66	24.40	12.95	25.38
	Sun, girdled	55.9	0.983	0.757	15.98	4.22	6.71	5.54	28.23	21.12	37.30
	Shade, girdled	59.2	0.977	0.718	4.02	4.93	8.42	6.32	18.76	5.59	26.12

¹ Below girdle in case of girdled trees.

character is determined more by the relations or qualities of foods than by the actual quantities present.

The low nitrogen content below the girdles may be associated with the poor root development on girdled trees rather than being a result of girdling, although the nitrogen content of the top is not related to root extension either on a percentage or absolute basis.

TABLE V
NITROGEN AND STARCH IN YOUNG APPLE TREES*

TREATMENT		NEW GROWTH	TWO YEAR WOOD		TWO YEAR ROOTS
			UPPER HALF	LOWER HALF	
Nitrogen	Sun	mg. 19.64	mg. 22.73	mg. 17.98	mg. 19.00
	Shade	11.36	32.68	30.18	23.45
	Sun, girdled.....	13.86	21.60	20.32	19.75
	Shade, girdled.....	16.75	29.30	8.98	8.68
Starch	Sun	165.7	237.8	169.2	332.1
	Shade	86.7	247.6	256.2	337.5
	Sun, girdled.....	177.1	331.9	209.6	417.0
	Shade, girdled.....	156.1	373.0	21.2	48.7

* The crown piece, including some stem and the main root as well as the new fibrous roots, are not included. Differences in previous season's bulk make direct comparisons of treatments useless, but the general tendencies where chemical differences are large can be clearly seen.

TABLE VI
EFFECT OF SHADE AND GIRDLING UPON THE PERCENTAGE OF BLOSSOM BUD FORMATION IN YOUNG APPLE TREES

TREATMENT	SPURS	TERMINALS	LATERALS	TOTAL BUDS
Sun	0.0	0.0	0.0	0.0
Shade	0.0	0.0	0.0	0.0
Sun, girdled	47.8	100.0	65.7	65.2
Shade, girdled	1.2	5.6	0.0	0.8

Two conditions will be noted from table VI. One is the relation of blossom bud formation to composition and growth character, and not directly to treatment. That is, girdled trees in the sun produced abundant blossom buds, but when such trees are shaded they are almost unfruitful as are the sun, ungirdled trees which have a like composition and growth character.

It will also be noted that blossom bud formation accompanied a condition of least growth and greatest carbohydrate content. This is because the very high carbohydrate, under-vegetative type of tree was not represented in the present series. The blossoming of trees of intermediate or "balanced" composition will be found in the following report upon the effect of reduced photoperiod upon apple tree performance.

It was desired to determine whether a "short day" had a direct effect upon blossom bud formation or produced its effects through changes in the plant composition. Consequently, trees which were in very different vegetative conditions due to previous variations in nitrogen nutrition were grown with both a full and a six-hour photoperiod from March to August, 1925. The trees were grown in the greenhouse in 12-inch earthen pots resting in trays for sub-irrigating. A ventilated "cage" constructed of slats and building paper was placed over the short day trees from 2 P. M. until 8 A. M. each day. Only six trees were grown in each lot. Because of the pronounced and consistent behavior of these trees (fig. 20), a record of their performance is given here.

The chemical composition on August 6, is shown together with the blossoming observed in early 1926, in table VII. Sampling was at approximate maturity of the long day plants. The short day trees with a high nitrogen nutrient definitely stopped vegetative extension only when water was withheld. They gave no sign of "maturing" as did the other trees.

There was a marked increase in length of growth. Variations in leaf and stem anatomy were striking. These will be reported in detail after repeating the photoperiod experiment with a larger number of trees.

Very clearly, a short photoperiod did not have the same blossom-forming effect upon the two lots of trees: it induced blossoming of the low nitrogen trees but entirely prevented blossom bud formation on the high nitrogen trees. Reproduction is here obviously related to the chemical composition rather than to the treatment and is certainly not directly induced by either a short or a long day. With a well balanced nutrition, apple trees are apparently "long day" plants, that is, they form blossom buds during the long days of early summer.

The long day-low nitrogen trees could be classed if desired as high carbohydrate, under-vegetative trees, and the short day-high nitrogen trees as low carbohydrate, over-vegetative trees. In a similar way the short day-low nitrogen and long day-high nitrogen trees could be spoken of as "balanced," fruitful trees. That is, fruitfulness accompanied a condition of intermediate amount of growth as well as nitrogen and carbohydrate content.

The behavior of the trees in a second season is shown by figs. 21 and 22. The removal of samples for analysis constituted a heavy pruning. The

TABLE VII
EFFECT OF PHOTOPERIOD UPON CHEMICAL COMPOSITION AND BLOSSOM BUD FORMATION IN DWARF APPLE TREES*

SAMPLE	DAY LENGTH	NITROGEN NUTRIENT	MOISTURE	TOTAL N	STARCH	REDUCING SUBSTANCES		ACID HYDROLYZABLE	TOTAL CARBO-HYDRATE	RATIO TO N		BLOSSOMS
						FREE	TOTAL			STARCH	CARBO-HYDRATE	
new growth	Long	Low ¹	Per cent. 46.1	Per cent. 0.70	Per cent. 8.08	Per cent. 2.43	Per cent. 5.25	Per cent. 7.01	Per cent. 20.34	11.5	29.1	Per cent. 0.0
	Short	Low	52.1	1.07	5.56	2.51	5.53	6.08	17.17	5.2	16.0	16.5
	Long	High	50.3	0.87	6.17	1.61	3.87	6.23	16.27	7.1	18.7	14.3
two year wood	Short	High	62.9	1.17	2.81	1.67	3.57	4.22	10.60	2.4	9.1	0.0
	Long	Low	48.5	0.43	10.91	2.96	4.94	7.81	23.66	25.4	55.0	
	Short	Low	50.2	0.55	5.74	1.78	4.83	6.86	17.43	14.4	31.7	
	Long	High	48.0	0.38	5.37	1.42	2.20	3.98	11.55	14.1	30.4	
	Short	High	51.0	0.60	3.04	1.52	2.94	4.73	10.71	5.1	17.8	

* Analyses by N. MOGENDORFF.

¹ Traces of NO₃ in tap water used. Other elements were supplied to the quartz sand used as soil.

effect of this operation upon new growth was noticeably less pronounced than the influence of light environment. Attention is especially called to fig. 21, showing the growth made by trees practically without nitrogen in the nutrient for four or five seasons. Two years of short-day treatment has produced a slender type of growth with large thin leaves which is characteristic of over-vegetative trees. That is, reduced period of illumination gave a type of growth wholly comparable to making heavy applications of nitrate to trees with full illumination. The nitrogen-fed trees in the short day for two years produced partially chlorotic foliage and an extremely weak spindly growth.

Before proceeding to a further discussion of the chemical and growth data, it is well to inquire as to what significance the analyses have. What value should be placed upon the chemical data?

The reducing substances extracted in hot concentrated alcohol usually show little relation to vegetative condition. This might well be expected in view of its presence being dependent upon variations in light and temperature as well as upon utilization.

Starch is the one carbohydrate being analyzed for which does usually appear to be closely related to growth condition. Starch values are obviously high in some samples as has already been pointed out (4). Very probably boiling the sample to gelatinize the starch extracts some reducing substance. Consequently the range in variation of starch content is really larger than is indicated by analyses.

A discouraging fact is the failure to secure correlation between the acid hydrolyzable fraction and vegetative condition. Miss BRADBURY (1) has pointed out that this is probably because the wall thickenings which are present in apple tree tissues and function as reserves are not hydrolyzed in the course of standard analysis. Because of the marked relation between vegetative condition and anatomical development, the "hemicellulose" fraction should be expected to show a clear correlation to plant growth. Here the analyses clearly do not indicate as large differences as exist between samples.

There is a large discrepancy in the amount of material extracted and that recovered as reducing substances. This is not only large but it is also variable, ranging from approximately 30 to 60 per cent. in the samples reported upon in table IV. This difference can not be explained by clarification precipitates, as clarification increased the reducing power of more than a majority of the 20 samples compared, table VIII.

Discussion

From these and other observations it is hardly necessary to say that a mathematical ratio between materials as nitrogen and the carbohydrate

TABLE VIII

EFFECT OF CLARIFICATION UPON THE PERCENTAGES OF STARCH AND ACID HYDROLYZABLE CONSTITUENTS OF SOME APPLE WOOD SAMPLES

SAMPLE	STARCH		ACID HYDROLYZABLE	
	CLARIFIED	UNCLARIFIED	CLARIFIED	UNCLARIFIED
One year wood. Check	7.40	7.12	6.05	3.81
Shade	6.61	5.74	3.73	4.21
Girdled	9.92	10.71	7.65	5.62
Girdled and shade	8.88	9.99	4.31	4.85
Two year wood. Check	5.82	4.26		
Shade	5.07	4.28		
Girdled	8.29	6.18		
Girdled and shade	7.40	6.82		
Two year roots. Check	14.20	15.88	4.29	3.77
Shade	12.42	11.74	5.66	4.78
Girdled	15.98	12.85	5.54	5.30
Girdled and shade	4.02	4.02	6.32	5.88

fractions is only an indication of relations. It might almost be asked if careful histological and micro-chemical records of qualitative differences would not better indicate the course of metabolism than do the macro-analyses when present standard methods are followed. Better methods of analyzing for the usual substances are needed as well as methods for other substances than are now considered.

Rather than suggesting that chemical data are of little importance because they show that different samples are not much different in composition, it seems to be a better interpretation to say that chemical data are often not of much value or significance because they represent only a part, too often small, of the real chemical differences existing between the samples, as is indicated by their marked differences in anatomical and growth character.

In addition to indications that blossom bud formation is related to a condition of "balanced" growth and composition, there is another significant situation which appeared in both series of trees. This is the relation of carbohydrate content or carbohydrate-nitrogen relation to vegetative elongation. This is shown particularly by the long growth of the short-day trees, especially those with a low nitrogen nutrient (fig. 21) and by the short growth of the girdled trees in the field.

How do very poorly vegetative trees become strongly vegetative by being placed in a short photoperiod? The change in percentage of nitrogen appears directly related to the fall in specific gravity due to a reduced carbohydrate content. With the respiration of carbohydrate material there is a

corresponding liberation of available nitrogen, as NIGHTINGALE (3) has found in the tomato. If carbohydrate respiration is a liberator of nitrogen forms which make for increased growth then carbohydrate accumulation must have previously been a binder of those nitrogen forms. Does carbohydrate accumulation inhibit vegetative extension? Do accumulating reserves check growth as well as accumulate after growth in length is checked? That could be an interpretation of the reduced growth of girdled trees. Girdling so changes other factors that this evidence might be weak. The relation of amount of elongation to composition in the shade-and-girdling series does, however, appear suggestive. The probable, if not apparent, relation of carbohydrate accumulation to reduced growth has many practical applications in connection with such questions as dwarfness, period of growth, "old age" in trees, partial etiolation, the rest period, as well as with blossom bud formation.

The "limiting factors" affecting growth may be within as well as without the plant. Clearly, relations rather than merely amounts of materials influence if they do not control vegetative response. The direct causes of growth responses should apparently be sought within the plant. At least, a fixed environment does not give a fixed response with plants of a different previous history. With agricultural perennials in a variable soil and climatic environment growth character should, then, be the basis for cultural treatment.

Summary

1. No separate environment had a consistent effect upon the composition, growth character or fruitfulness of young apple trees. For example, girdling checked growth and induced fruiting; shading of girdled trees neutralized the effects of girdling giving a growth situation comparable to ungirdled sun trees. Again, short-day trees without nitrogen grew and blossomed like long-day trees with nitrogen.

2. Growth character, including blossom bud formation, is primarily dependent upon internal composition and secondarily upon external environment. That is, of the five environmental conditions employed—sun, shade, girdling, photoperiod, nitrogen nutrient—none produced a specific growth situation.

3. The type of growth should be the condition used as a basis for deciding upon cultural practices.

4. Chemical analyses as developed at present provide only a limited and not too accurate method of measuring plant performance.

HORTICULTURAL DEPARTMENT,
UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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EXPLANATION OF PLATES

PLATE II. Figs. 2-5. Upper epidermis of sun, shade, sun and girdled, and shade and girdled leaves. Camera diagrams by MARIAN DEATS ABEGG.

Figs. 6-9. Lower epidermis of sun, shade, sun and girdled, and shade and girdled leaves. Camera diagrams.

Figs. 10, 11. Camera diagrams of cross-section of leaves of sun and shade leaves.

PLATE III. Figs. 12-15. Camera diagrams of cross-section in pith of new wood: sun, shade, sun and girdled, and shade and girdled.

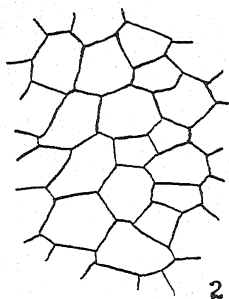
Figs. 16, 17. Pith cross-section in lower stems of sun and girdled and sun trees. Note utilization of starch and wall thickening reserves in 16.

PLATE IV. Figs. 18, 19. Cross-section diagram in new growth wood of shaded and sun and girdled trees.

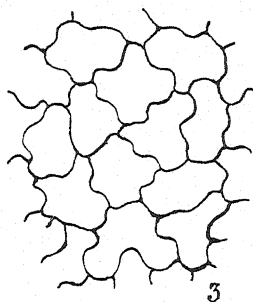
Fig. 20. Trees with nitrogen. Left, long day; right, short day.

PLATE V. Fig. 21. Trees without nitrogen. Center, long day, two years; left, short day in 1925 and long in 1926; right, short day, two years.

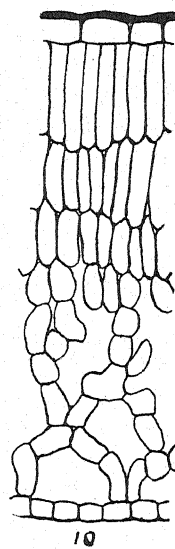
Fig. 22. Trees with nitrogen. Right, short day in 1925, long day, 1926; left, short day, two years. Note the seasonal change in foliage character at left.



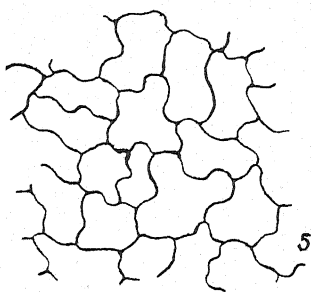
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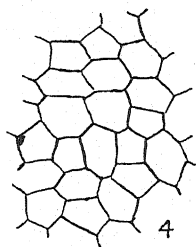
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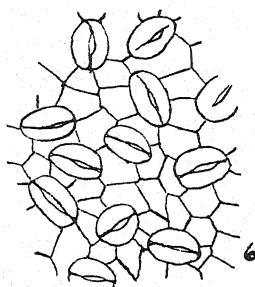
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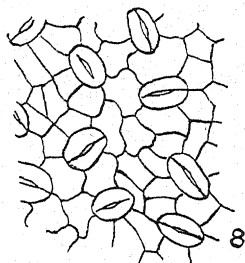
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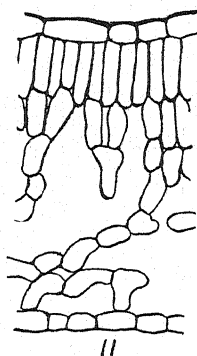
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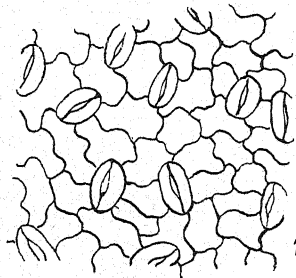
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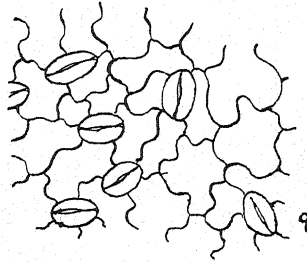
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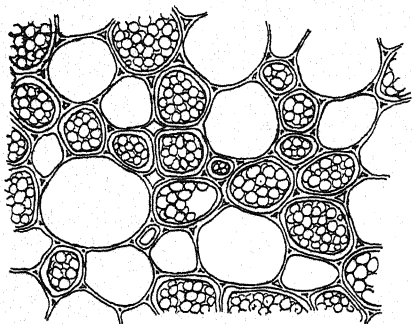
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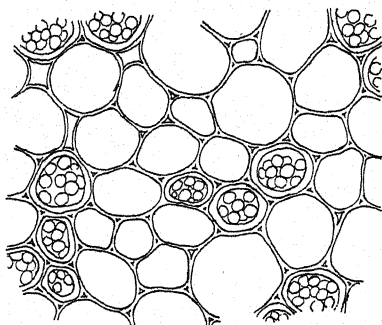
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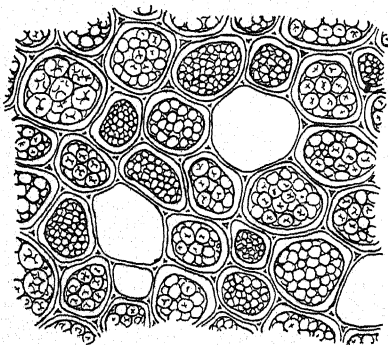
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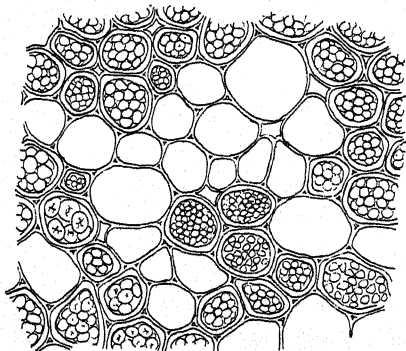
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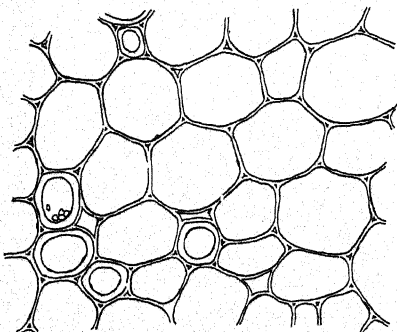
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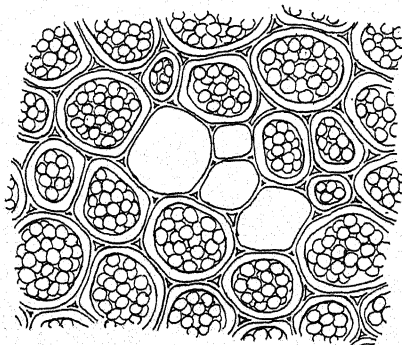
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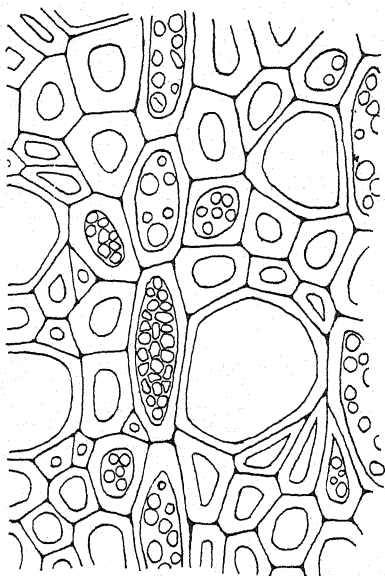
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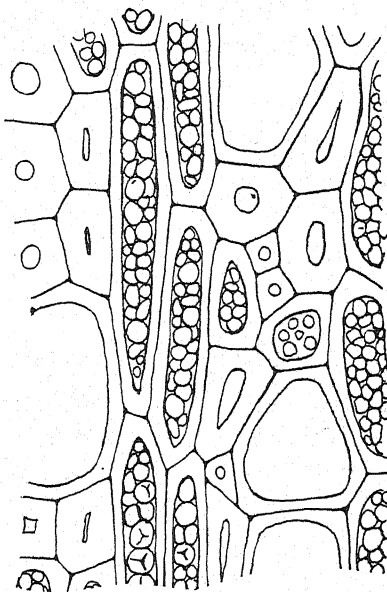
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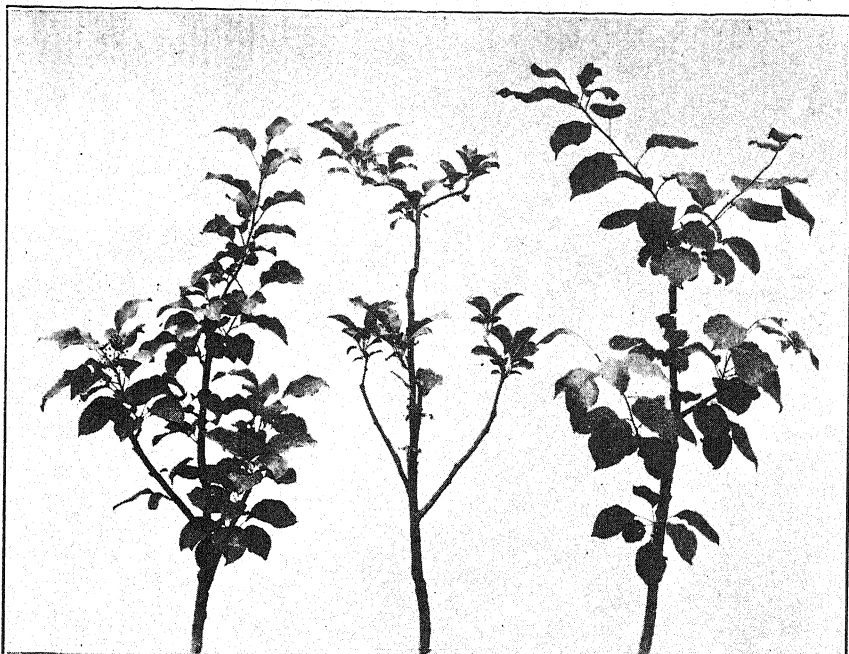


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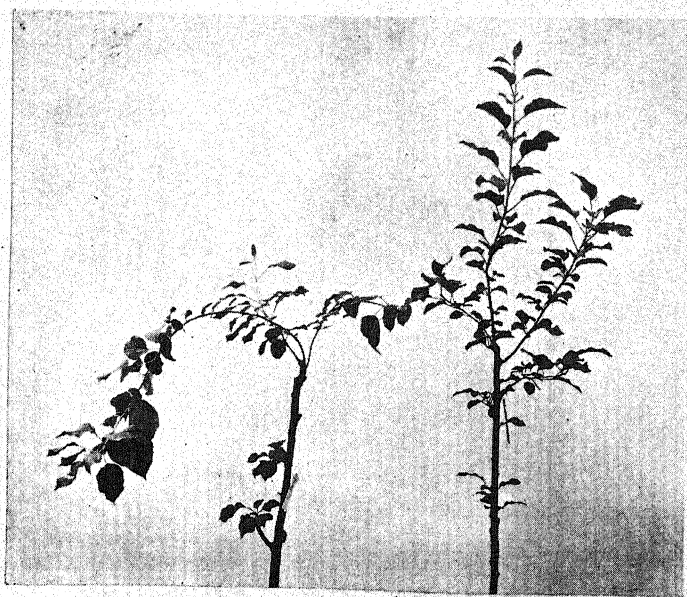


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22

TEMPERATURE AND OTHER FACTORS AFFECTING THE REST PERIOD OF POTATO TUBERS¹

W. E. LOOMIS

(WITH FOUR FIGURES)

The rest period and means of breaking the rest period of seeds and stems are of very general scientific interest to plant physiologists. Some means of breaking the rest period of the irish potato is also of economic importance in those sections of the country where late varieties cannot be grown successfully, and where the high summer temperature limits the common storage period of the early crop to about three months. The fall crop of these early varieties supplies the best keeping potatoes obtainable in the South, but northern grown seed cannot conveniently be held until the late July or early August planting date, and seed from the spring crop does not germinate with usual treatments in time to produce a second crop the same season. The result is that the South either has to import 50 million bushels of potatoes or resort to less satisfactory substitutes for this important food product. At 1926-27 prices, one-fifth of the cotton produced in the older southern states would be used in exchange for potatoes if the average consumption of the country were maintained in this section.

Methods used to shorten the rest period of potatoes

A number of workers have employed chemicals of various kinds to break or shorten the rest period of potato tubers. Of these McCALLUM (6) recommended ethyl bromide; APPLEMAN (2) used hydrogen peroxide; ROSA (9) employed nitrate of soda and later (10) ethylene gas; DENNY (5), in a large number of trials, found the ethylene derivatives, ethylene chlorohydrin and ethylene dichloride, to be very effective in breaking the rest period of irish potatoes. With the exception of the nitrate of soda treatment, which is not always effective, all of these methods require special chemicals and equipment, and a skill in manipulation which is not always available in commercial practice.

Among the workers who have resorted to nonchemical means of shortening the rest period, MÜLLER-THURGAU (7) was able to force potatoes in one

¹ Published with the consent of the Director as paper no. 52 of the Journal series of the University of Arkansas.

month by storing them at 0° C. In a later paper (8) he reported negative results with a "warmbath" treatment at 35° C. for one hour. APPLEMAN (2) found that treatments which facilitated the exchange of oxygen between the tuber and the atmosphere were highly effective in breaking the rest period of freshly dug tubers. In order of effectiveness he recommended peeling, covering of immature tubers so that a subdued light reached them, and cutting of mature or immature potatoes. The effectiveness of the treatments was reduced by allowing the injured surfaces to dry and by covering the treated tubers with soil instead of moss or excelsior. For these reasons his methods are not easily applied commercially.

STEWART (12) describes six practices as in use in various sections of the South. 1. The cull tubers are thrown back into the row at digging time and re-covered immediately. 2. The culls are placed in a shallow trench and covered with moist earth until planting time, usually about one month later. 3. The tubers are spread out in diffused light and allowed to green. 4. The tubers are spread as in three but are covered with straw and kept moist. 5. The tubers are placed in cold storage at 32° F. for three or four weeks and allowed to warm up before planting. 6. Pieces of skin are clipped from the small tubers which are to be planted whole. This practice may be combined with any of the others. CORBETT (4) favored a preliminary dry storage plus two weeks of treatment no. 4.

Experimental work

The work reported here considers the more practical aspects of a study of irish potato dormancy which was begun at the University of Arkansas in 1925, and which we hope to continue to a more satisfactory explanation of the phenomena involved. Both the tables and discussion are given as briefly as possible and as a result some details of manipulation are omitted. While no attempt was made in most of the experiments to secure exact control of growing temperature, moisture, etc., every precaution was taken to obtain comparable conditions by arrangement of flats, repetitions, mixing of soil, careful watering by the author, selection of equally sized tubers, etc. In experiments involving treatments given at or after planting time, pieces from the same tubers were used in each of the comparisons whenever this was possible. Unless otherwise specified, sound, disease-free tubers weighing between 3.75 and 4.25 ounces were quartered longitudinally and not more than one piece from a tuber used in a given treatment. Thus in the experiments using 40 pieces per lot, 40 tubers are represented.

The data given may conveniently be considered under three groupings: (1) Factors which affect the degree of dormancy at the time the potatoes are dug, or seed factors. (2) Factors which influence the rest period during

the interval before the crop is replanted, or storage factors, and (3) Factors which influence the germination of the planted potatoes, or soil factors.

SEED FACTORS AFFECTING DORMANCY

VARIETY.—It is a common observation that some varieties exhibit a more profound dormancy than others. Of the two varieties used in this work, Bliss Triumph responded more readily to both storage and chemical treatments than Irish Cobbler. No extensive variety tests were made but the author suggests, in the light of other experiments and analyses, that those varieties classed as mealy because of their normally high starch content may show deeper dormancy than the varieties having a lower starch and higher protein content. It may be noted in this connection that potatoes grown under high moisture and temperature conditions frequently exhibit so little dormancy as to sprout before maturity and while the parent vine is still green. BUSHNELL (3) has shown that there is a deficiency of carbohydrates in potato plants grown under these conditions.

FERTILIZATION.—The influence of the fertilizers applied to the growing crop upon the dormancy of the tubers produced was observed briefly. The data in table I indicate that there was a slight correlation between fertilization and the germination of the tubers produced. Unfortunately the experiment does not distinguish between the effects of nitrogen and potash and so can be only suggestive for future work in which the relation of nitrogen to the accumulation of carbohydrates, and of the K-Ca balance to permeability will be differentiated.

TABLE I

EFFECT OF PREVIOUS FERTILIZATION UPON THE DORMANCY OF POTATOES

SEED TREATMENT AND PREVIOUS FERTILIZER APPLICATION	PIECES SPROUTED	SPROUTS UP	TOTAL WEIGHT SPROUTS
	Per cent.	Per cent.	gm.
Seed treated with ethylene			
No fertilizer	97.5	42.5	61.3
1200 pounds 4-8-4 fertilizer.....	100.0	75.0	61.2
1200 pounds 4-0-4 fertilizer.....	100.0	87.5	70.9
Seed untreated			
No fertilizer	67.5	67.5	6.5
1200 pounds 4-8-4 fertilizer.....	97.5	67.5	7.0
1200 pounds 4-0-4 fertilizer.....	92.5	75.0	9.5

SIZE OF TUBER.—Extended observations indicated that the larger tubers in a given lot of potatoes have a shorter rest period and respond more readily

TABLE II
RELATION OF TUBER SIZE TO RATE OF GERMINATION OF DORMANT POTATOES

DATE OF PLANTING	VARIETY	PREVIOUS TREATMENT	ONE OUNCE PIECES CUT FROM 4 OZ. TUBERS		CLIPPED, ONE OUNCE TUBERS	
			GERMINATION	TOTAL WEIGHT SPROUTS	GERMINATION	TOTAL WEIGHT SPROUTS
October 21, 1925.....	Triumph	None	Per cent.	gm.	Per cent.	gm.
December 2, 1925.....	Triumph	18° storage	28.0	7.3	0.0	0.0
December 2, 1925.....	Cobbler	18° storage	8.0	0.4	0.0	0.0
December 2, 1925.....	Triumph	1° storage	13.3	7.7	0.0	0.0
December 2, 1925.....	Cobbler	1° storage	84.0	87.6	76.7	81.7
December 2, 1925.....	Triumph	28° storage	10.0	51.5	20.0	22.0
December 2, 1925.....	Cobbler	28° storage	94.0	147.9	56.6	50.5
December 2, 1925.....	Triumph	28° storage	73.3	41.7	56.6	6.3
November 9, 1926.....	Cobbler	None	80.0	11.0	20.0	1.5
November 9, 1926.....	Triumph	None	95.0	7.9	35.0	2.6
November 9, 1926.....	Triumph	0.8% ethylene	70.0	239.0	17.5	21.1
December 23, 1926.....	Triumph	20° storage	100.0	78.8	90.0	18.8
December 23, 1926.....	Triumph	20° storage	100.0	75.2	85.0	24.3
Average			63.0	63.8	38.1	19.1

to various treatments than do those of smaller size. The difference appears to be sufficiently large and consistent to justify the recommendation that only tubers weighing three ounces or more be used for second crop seed where rapid germination is desired. The smaller sizes germinate as readily as the larger when both are held in storage for several months before planting, and the difference seems to be one of development or maturity.

In the experiments reported in table II, one ounce seed pieces were cut from four ounce tubers by longitudinal sectioning, and one ounce tubers were partially peeled to obtain a comparable wound stimulus. In the two series of experiments reported, the first planting date was immediately after harvest. The second planting date shows the duration of the storage condition indicated in the third column. Fifty tubers are represented in each lot in the 1925 experiments and 20 in those run in 1926. A 65 per cent. better germination and 100 per cent. greater growth per germinating sprout is shown for pieces of the same size cut from the larger tubers. Two ounce tubers gave intermediate results. The 1925 plantings were harvested on Feb. 11, 1926, and the 1926 plantings on Feb. 21, 1927.

STORAGE FACTORS AFFECTING THE DORMANCY OF POTATOES

Storage treatment for shortening the rest period of potatoes, particularly if they are such as can be given on the average farm, are of especial interest from the practical viewpoint. APPLEMAN (2) has stressed suberization as a factor contributing to dormancy and he found that the suberization of immature tubers could be retarded or prevented by placing them immediately in moist excelsior. MÜLLER-THURGAU (7) was able to break the rest period of irish potatoes by storage at 0° C. The two factors, humidity and temperature, thus seem to be involved, and an extensive study was made of their separate and combined effects.

HUMIDITY.—Nineteen comparisons were made in which various lots of potatoes were stored at various temperatures in air and damp moss. The temperature of the potatoes stored in moss was normally 2–4° below that of the chamber and this temperature difference probably accounts for some of the differences in growth response which were observed. When the storage temperature was moderate the dry potatoes gave the best germination; when it was high those stored in moss showed the least injury and made the best growth. The beneficial effect of the moss lay apparently in the conservation of moisture rather than in the reduction of suberization, and is in all probability related to the slow formation of wound periderm which SHAPOVALOV and EDSON (11) observed in shriveled tubers.

TEMPERATURE.—In the first experiments on storage temperature it was assumed that storage at 0° or 5° C. would give the best results and higher

temperatures were used only as checks. One lot of tubers was accidentally left over a steam radiator and was badly shriveled at the end of ten days. When planted, this lot surprisingly gave the best germination of any of the storage treatments used and was also superior to any of the lots treated with various salt solutions, including nitrates and sulphates. Continued experiments involving a hundred and eighty lots of potatoes have very clearly shown a shortening of the rest period by storage temperatures around 30° C. The optimum storage temperature varies with the size and the condition of the tubers used and the duration of the experiment, but a temperature of 30°–33° C. for a period of four weeks has given generally satisfactory results with 4 ounce tubers. Similar effects were obtained whether the tubers were stored in an incubator at a constant temperature or in the attic of a low roofed building having an average temperature of approximately the same degree, so that the commercial application of the method during the southern summer is a simple matter.

The experiments on storage temperature were run in three series, employing the fall crop of 1925 and the summer and fall crops of 1926. In 1925 Triumph and Cobbler, second crop tubers were harvested on October 20 immediately after the first killing frost. The check lots were planted and the storage treatments started on the following day. The checks were thus planted on October 21, the three weeks' storage lots on November 11, and the six weeks' storage lots on December 2. All treatments were harvested together on February 11 so that the growing periods for the three plantings were 112, 92, and 71 days respectively.

The potatoes stored at 16°–20° were packed in damp moss, while the others were in dry storage. The treatments marked 20°–35° were intended to have been 20°–23° and the high temperature during the second week was accidental. In addition to the irregularity of the temperature on these lots, some of them were injured by excessive temperatures.

Because of the preliminary nature of the work complete data are not given, but an average of the check and six weeks' storage treatments is included in table III. Five lots of forty pieces are averaged for each figure. The table indicates the conclusion which was drawn from the series, namely, that moist storage at moderate temperatures was no more effective than immediate planting in breaking the rest period and that, in spite of the temperature irregularities and injury to some tubers, dry storage was slightly more effective than cold storage at 0°–2° C.

In the second series, potatoes dug July 1, 1926, were held at 20° C. until July 6–8, when the check plantings were made and storage lots selected. Four storage conditions were used for four weeks' storage periods, namely, commercial storage at 0°–2° C., cellar storage at 20°–21° C., an insulated room at 23°–28° C. and an attic room beneath a low roof which had a tem-

TABLE III
EFFECT OF STORAGE TEMPERATURE UPON THE DORMANCY OF IRISH POTATOES
SERIES I

STORAGE TREATMENT FROM OCT. 21 TO DEC. 2	SPROUTS THROUGH ON FEB. 11	PIECES GERMINATED	AVERAGE TOTAL WEIGHT SPROUTS FROM 50 PIECES
	Per cent.	Per cent.	gm.
None (planted Oct. 21)	0.5	7.3	1.5
Damp moss at 18° C.	0.7	4.3	1.0
Cold storage at 1° C.	22.2	55.7	62.7
Heated room at 20°-35° C.	17.1	72.1	57.3

perature of 25°-35° C. Average temperatures for the two rooms were roughly 25° and 30°, while the cellar temperature was very constant at 20° C. Tubers stored for four weeks at the four temperatures were planted August 4 in deep flats filled with a sandy soil, and duplicate lots held at 20° and at approximately 25° to observe the germination rate. Forty one-ounce pieces, representing forty 4-ounce tubers, were used in each lot. The remaining pieces from these tubers were treated with 2 per cent. ethylene chlorohydrin by a method very kindly furnished to the author by Dr. F. E. DENNY, of Boyce Thompson Institute, and were placed with the checks at the two germination temperatures.

TABLE IV
EFFECT OF STORAGE TEMPERATURE UPON THE DORMANCY OF IRISH POTATOES
SERIES II

STORAGE TREATMENT FROM JULY 7 TO AUGUST 4	GERMINATION TEMPERATURE FROM AUG. 4 TO AUG. 31	SPROUTS UP ON AUG. 23	PIECES GERMINATED ON AUG. 31	TOTAL WEIGHT SPROUTS FROM 40 PIECES AUG. 31
		Per cent.	Per cent.	gm.
None (planted July 7)	20° C.	0.0	5.0	0.2
Cold storage—1° C.	20° C.	25.0	82.5	4.3
Cellar—20° C.	20° C.	16.3	90.0	4.6
Room—25 ± 3° C.	20° C.	43.8	85.0	8.3
Attic—30 ± 5° C.	20° C.	68.8	88.8	13.8
None (planted July 7)	25° C.	87.5	7.0
Cold storage—1° C.	25° C.	37.5	52.5	5.5
Cellar—20° C.	25° C.	60.0	97.5	9.8
Room—25 ± 3° C.	25° C.	66.3	95.0	12.8
Attic—30 ± 5° C.	25° C.	100.0	100.0	16.0

A comparison of the growth made by the tubers stored for four weeks at 30° C. with the growth made by those treated with 2 per cent. ethylene chlorohydrin is given in table V. The ethylene treatment was most effective on the lots from the lower temperatures and had very little influence on the tubers from 30° storage, particularly at the higher germinating temperature, thus indicating that these lots had been brought through the rest period in four weeks. Tubers treated with ethylene on July 7 made a much better growth than the check as is shown in tables I and IX, but it is normally desirable to hold Triumph potatoes from digging time in June until the latter part of July or first of August in order to obtain the maximum benefit from the cool fall weather, so that the potatoes are actually stored for 4 to 6 weeks and the high temperature storage treatment is easily added.

Bliss Triumph potatoes planted August 1 and dug November 6 were used for the third series which was started November 8, 1926. Carefully selected lots of twenty or more tubers were stored in a series of incubators. For the lower temperatures the incubators were set up in a cold storage room. As originally planned the series was arranged in 5° intervals from 0° to 35° C., but the failure of the thermostat in the 10° chamber and injury from a mercury disinfectant used on the 25° and 30° lots reduced the comparisons finally used to those given in table VI, and illustrated in figures 1 and 2.

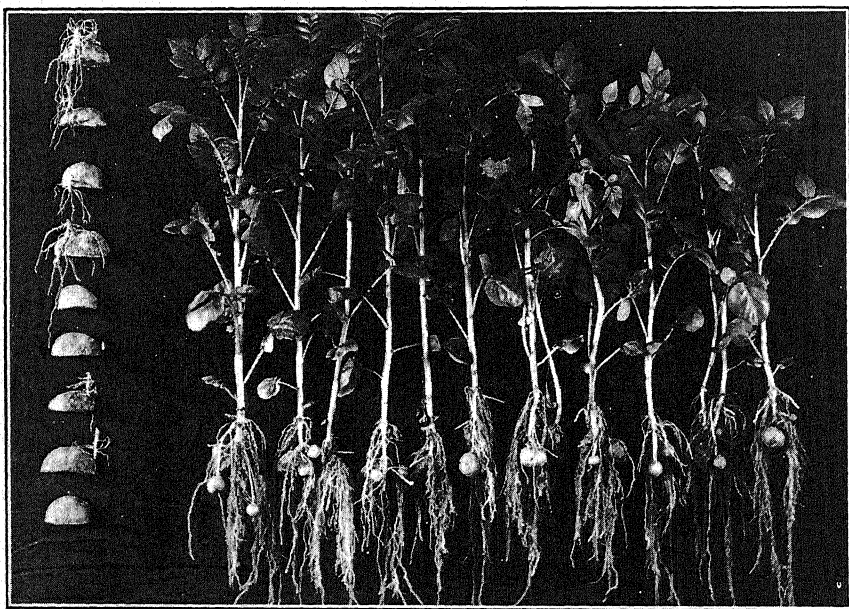


FIG. 1. Effect of storage at 35° C. upon the dormancy of potatoes. Left, planted November 8, 1926, and photographed February 21, 1927, 105 days after planting; right, stored November 8 to December 23 at 35° C. and photographed 60 days after planting.

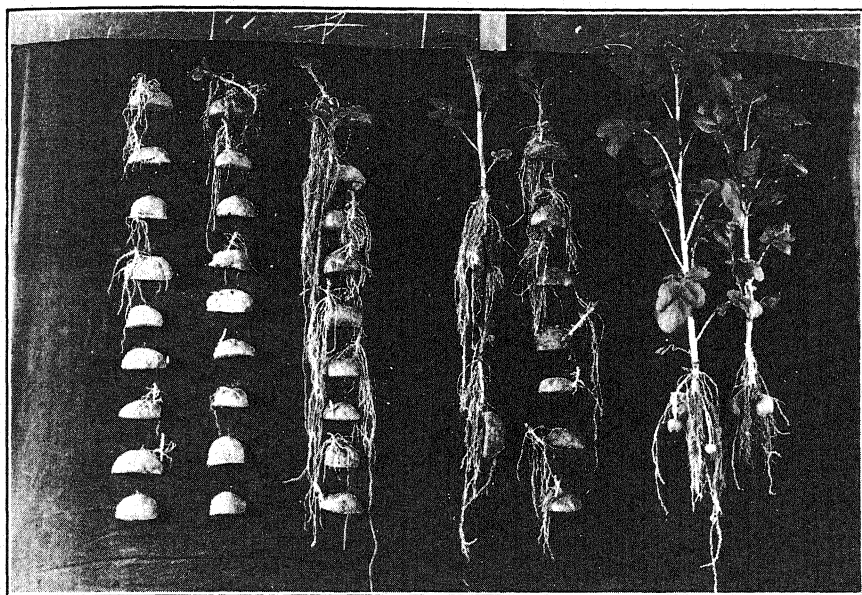


FIG. 2. Increased growth with increased storage temperature. Left to right in vertical arrangement, check planted November 8, 1926; stored at 5° C. until December 23; stored at 15° C.; stored at 20° C.; and representative plants from the tubers stored at 35° C. Photographed on February 21, 1927.

TABLE V

COMPARISON OF ETHYLENE CHLOROHYDRIN AND HIGH STORAGE TEMPERATURES FOR FORCING DORMANT POTATOES

TREATMENT	GERMINATED AT 20° C.		GERMINATED AT 25° C.	
	GERMINATION	WEIGHT SPROUTS PER 40 PIECES	GERMINATION	WEIGHT SPROUTS PER 40 PIECES
	Per cent.	gm.	Per cent.	gm.
30° storage	88.8	13.8	100.0	16.0
30° + ethylene	100.0	18.5	96.2	17.0
20° storage	90.0	4.6	97.5	9.8
20° + ethylene	95.0	19.8	91.2	18.0
Average all ethylene treatments.....	98.5	18.9	95.0	18.9

SOIL FACTORS AFFECTING THE DORMANCY OF PLANTED POTATOES

MOISTURE.—Moisture may be a limiting factor in the germination of second crop potatoes, particularly when they are planted on the land occupied

TABLE VI
EFFECT OF STORAGE TEMPERATURES UPON THE DORMANCY OF IRISH POTATOES
SERIES III

STORAGE TREATMENT FROM Nov. 8 TO DEC. 23	RECORD FROM 40 PIECES ON FEB. 21			
	SPROUTS THROUGH	PIECES GERMINATED	TOTAL GREEN WEIGHT TOPS	TOTAL WEIGHT NEW TUBERS
	Per cent.	Per cent.	gm.	gm.
Check (planted Nov. 8).....	17.5	87.5	18.8	0.0
Incubator at 5° C.	27.5	82.5	41.8	3.8
Incubator at 15° C.	50.0	100.0	55.2	0.0
Incubator at 20° C.	85.0	100.0	154.0	21.6
Incubator at 35° C.	77.5*	77.5*	855.2	252.1

* Six weeks' storage at 35° was more than was necessary for the 4 ounce tubers and they showed injury which resulted in rot after the pieces were planted. Small tubers stored at this temperature were less shriveled and gave perfect germination but a smaller total growth.

by the first crop. Field observations in 1926 suggested that a secondary dormancy might be induced in chemically treated seed by planting in soil whose moisture content is too low for immediate germination. Under most conditions, however, moisture is more likely to be a limiting factor in yield per hill than in germination. Thus in the tests referred to above, second crop potatoes planted after fallow came up only to a 26 per cent. better stand than those planted after early potatoes; but they produced a 50 per cent. better yield per hill and an 84 per cent. better yield per acre than the potatoes on the double cropped land.

FERTILIZER.—The stimulating effect of nitrates and certain other salts, particularly sulphates, upon the germination of dormant Irish potatoes suggested that row fertilization might be a factor in the germination of second crop potatoes under field conditions. A large number of experiments were run in which cut, partially dormant tubers were soaked in salt solutions of varying strengths. Sodium nitrate, sodium and potassium sulphate but especially ammonium sulphate gave a better growth than the checks. In pot and field experiments potassium sulphate gave a more rapid germination than potassium chloride, and ammonium sulphate than nitrate of soda or dried blood. Attempts to force freshly dug tubers with ammonium sulphate used as a fertilizer were less successful than the soaking experiments because of the toxic effect of the heavier applications. The toxic effect of the sulphate was, however, less than that of nitrate and it would seem to be the more desirable source of nitrogen for second crop potatoes.

In some of the early experiments, sand was compared with soil as a germinating medium for dormant tubers on the assumption that the greater aeration in the coarse sand would be advantageous. The pieces planted in soil, however, germinated more rapidly and made a greater growth than those in sand. This growth was further increased by soaking the seed pieces in M/10 nitrate solution or by adding composted manure to the soil, so that the increase would appear to be due to nitrogen fertilization. The data in table VII indicate that an increased supply of nutrients increased both sprout and root growth, but the latter more rapidly than the former. The seed used was northern grown Triumph dug about October 1 and planted December 16 when they were still dormant but largely out of the rest period. Records were taken on January 17 when the sprouts from the pieces planted in soil were just coming through. Tubers from the same lot were used in a second experiment in March after they had begun to sprout, with the result that the pieces planted in a poor, sandy soil made the best growth, followed in order by those fertilized with ammonium sulphate, acid phosphate and potassium sulphate, manure compost, and nitrate of soda. In field experiments also, row or heavy broadcast fertilization has commonly delayed the germination of potatoes fully out of the rest period.

TABLE VII

GERMINATION OF PARTIALLY DORMANT TUBERS IN SAND, SOIL, AND A SOIL AND MANURE COMPOST

SEED TREATMENT	GROWING MEDIUM	TOTAL WEIGHT FROM 40 PIECES		RATIO OF ROOTS TO TOPS
		SPROUTS	ROOTS	
		gm.	gm.	Per cent.
None	Sand	61.3	26.1	42.6
Soaked in M/10 nitrate.....	Sand	69.5	41.2	59.3
None	Soil	79.1	35.9	45.4
Soaked in M/10 nitrate.....	Soil	96.1	57.4	59.7
None	Compost	184.9	124.5	67.3
Soaked in M/10 nitrate.....	Compost	192.6	123.3	64.0

In another experiment, second crop tubers dug November 6 were divided into three lots, one of which was planted immediately in deep flats filled with sand and with composted soil while the other two were stored until December 23 at 20° and 35° C. They were then planted in sand and in compost until January 25. The greater growth of the stored lots when planted in compost is shown in figure 3, and the complete data are given in table VIII. Apparently the nutrients furnished by the compost stimulated the germination of the dormant tubers and increased the growth of those partially dormant.

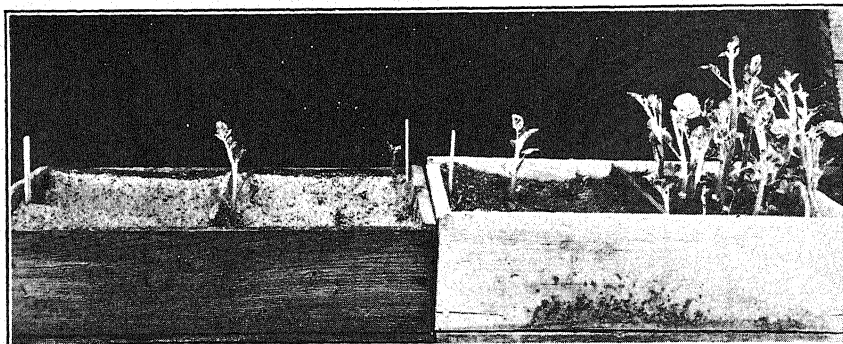


FIG. 3. Soil and temperature effects on the germination of dormant potatoes. Flat at left filled with sand and at right with soil and manure compost. Tubers in left half of each flat stored at 20° C. for six weeks before planting and those in right half at 35° C. Photographed 35 days after planting.

TABLE VIII

GERMINATION OF DORMANT AND PARTIALLY DORMANT TUBERS IN SAND AND COMPOST

STORAGE TREATMENT Nov. 9 TO DEC. 23	GROWING MEDIUM	SPROUTS UP ON JAN. 17	PIECES GERMINATED	TOTAL WEIGHT SPROUTS FROM 25 PIECES
		Per cent.	Per cent.	gm.
None (planted Nov. 9).....	Sand	0.0	0.0	0.0
None (planted Nov. 9).....	Compost	25.0	52.5	83.6
Incubator at 20° C.	Sand	0.0	24.0	1.9
Incubator at 20° C.	Compost	16.0	52.0	13.1
Incubator at 30° C.	Sand	76.0	100.0	72.1
Incubator at 30° C.	Compost	88.0	100.0	199.7

SOIL TEMPERATURE.—If high storage temperatures shorten the rest period, it should follow that high soil temperatures would favor the continuation of the same processes and be equally beneficial in starting the growth of dormant potato tubers. Such has been found to be the case. The average growth of the treatments in table IV which were germinated at 25° C. is 60 per cent. greater than the average of those held at 20° C., while the growth of the 25° check is several hundred per cent. better than that of the 20° check. The data in tables IX and X indicate that the effect of high soil temperatures applies to freshly dug tubers treated with ethylene chlorohydrin, and that it may be more important than the storage temperature, with untreated lots (see figure 4). Potatoes dug July 1, 1926, were planted, with and without ethylene treatment on July 7 and placed under the pre-

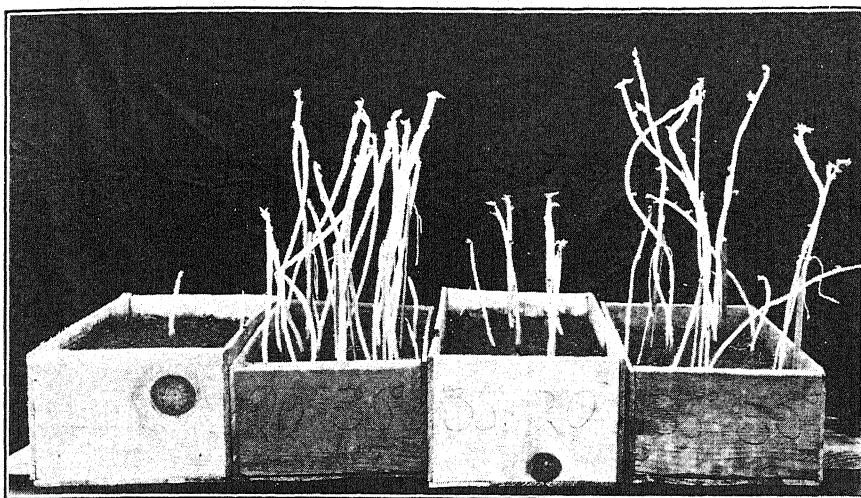


FIG. 4. Effect of storage and germination temperatures upon dormant potatoes. Left to right, stored 6 weeks at 20° C. and germinated 4 weeks at the same temperature; stored at 20° and germinated at 32°; stored at 35° and germinated at 20°; stored at 35° and germinated at the same temperature.

viously described conditions in a cellar insulated room, and low-roofed attic. The treated lots were harvested on July 26, and the checks on August 31. Thermograph records showed that the cellar temperature was very constant at 20° C., that the temperature of the room fluctuated around 25° C., lowering somewhat toward the end of the period, and that the attic temperature was roughly 30° C. for July and 25° C. for August.

TABLE IX

RELATION OF SOIL TEMPERATURE TO THE GERMINATION AND GROWTH OF DORMANT AND TREATED POTATO TUBERS

TREATMENT	APPROXIMATE GROWING TEMPERATURE	SPROUTS UP AT HARVEST	PIECES GERMINATED	TOTAL GREEN WEIGHT SPROUTS FROM 40 PIECES
		Per cent.	Per cent.	gm.
None	20° C.	2.5	5.0	0.2
None	24° C.	40.0	80.0	4.5
None	28° C.	60.0	87.5	7.0
2 per cent. ethylene.....	20° C.	12.5	60.0	8.5
2 per cent. ethylene.....	25° C.	70.0	77.5	44.9
2 per cent. ethylene.....	30° C.	82.5	92.5	82.1

A second experiment was run in the fall of 1926 in which tubers stored in incubators at 20° C. and 35° C. for six weeks were replaced in incubators at 20° C. and 32° C. after planting, both storage temperatures being represented at each germination temperature. Six weeks' storage at 35° C. was excessive for this material and rot loss greatly reduced the showing of the tubers from the high temperature storage. Those pieces which germinated were, however, ahead of the pieces previously stored at 20° C. and made a 24 per cent. greater growth per growing sprout. The final growth on this experiment is shown in figure 4, while germination and growth data are given in table X.

TABLE X

RELATION OF SOIL TEMPERATURE TO THE GERMINATION OF PARTIALLY DORMANT POTATO TUBERS

STORAGE TEMPERATURE	GERMINATION TEMPERATURE	ROTTED	GERMINATED	TOTAL WEIGHT SPROUTS FROM 25 PIECES
		Per cent.	Per cent.	gm.
20° C.	20°	0.0	60.0	9.1
35° C.	20°	68.0	32.0	38.4
20° C.	32°	8.0	88.0	165.4
35° C.	32°	60.0	40.0	92.8

A comparison of the growth at 20° and 25° of potatoes treated immediately after digging (table IX) and potatoes treated a month after digging (table V) suggests that the effect of the high temperature was to complete the breaking of the rest period. Tubers treated immediately after digging made a four hundred per cent. better growth at the higher germinating temperature while there was no difference in the lots treated with ethylene four weeks after digging.

Discussion

The work reported in this paper harmonizes with previous publications on the subject of dormancy in that the successful treatments have been those which increase respiration. The observations on the possible stimulating effects of nitrogenous compounds afford an interesting basis for speculation upon the relation of starch hydrolysis and protein synthesis to dormancy and growth. It is insufficient, however, to state that we are concerned here with an application of the law of carbohydrate: nitrogen balance since the addition of nitrogen and an extended period of high respiration are conditions favoring, rather than requirements for, the breaking of the rest period of potato tubers. A study of nitrogen distribution offers more promise and our preliminary chemical studies have shown important shifts

in the colloidal and non-colloidal nitrogenous fractions. Such changes are, however, more probably the result of sugar accumulations from hydrated starch than the cause of this reaction. APPLEMAN (1, 2) was unable to correlate the rest period with enzyme changes, although he found sugar accumulation characteristic of changes which took place toward the end of the rest period when the tubers were stored at low temperatures. It would seem that dormancy is more probably related to cytoplasmic structure than to chemical composition or enzyme formation and that the rest period is broken by some such change as the reversal of lipoidal, proteinaceous phases in the cytoplasm whereby the permeability of the cell is increased and the enzymes associated with the protein phase are liberated. Such a theory is in harmony with the efficiency of fat solvents such as ether and ethylene in shortening the rest period and with the more rapid rate of water loss from tubers which are out of dormancy, but is not easily demonstrated experimentally.

Summary

Experiments are reported in which the rest period of dormant irish potato tubers was broken in four weeks by storage during July in the attic of a low-roofed building. Extended studies have established the efficacy of storage temperatures around 30° C. in shortening the dormant period of potato tubers and have demonstrated the importance of high soil temperatures for the rapid germination of partially dormant tubers.

Three and four ounce tubers selected from a given lot of freshly dug potatoes have germinated more readily and made a stronger growth than one and two ounce tubers from the same lot. The difference is lost as the tubers pass through the rest period.

Composted soils and low concentrations of nitrogenous fertilizers had a stimulating effect on the germination of dormant and partially dormant tubers which was not observed in tubers which were completely out of the rest period.

Storage in damp moss, as compared to dry storage, at high temperatures has reduced shriveling and rot loss subsequent to planting, presumably because of the more rapid formation of a wound periderm in the turgid tubers, but has had no direct effect upon the rest period.

DEPARTMENT OF HORTICULTURE,
UNIVERSITY OF ARKANSAS.

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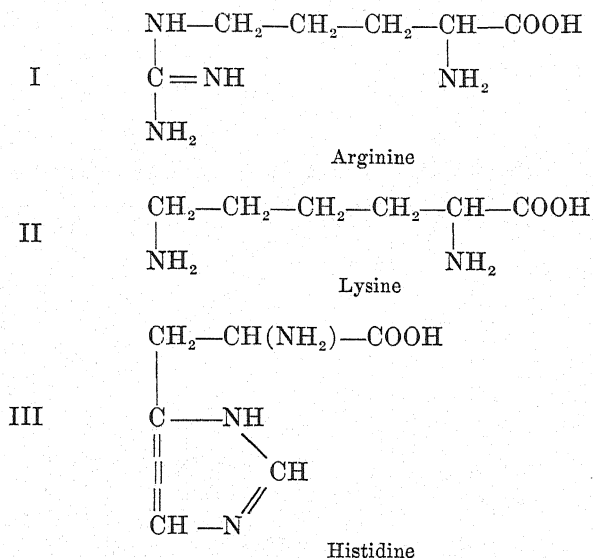
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THE BASIC NITROGEN OF PLANT EXTRACTS*

HUBERT BRADFORD VICKERY¹

The estimation of the proportion of the nitrogen of a plant extract which occurs in the form of simpler nitrogenous bases is a problem of great importance. Although the physiological significance of many of these substances can, at present, only be guessed at, there is no doubt that they play an important part in metabolism, and if adequate information could be obtained it is probable that many relationships which are now obscure might become clear.

It is useful to distinguish those of the simpler bases which bear a direct relationship to the amino-acid constituents of proteins from other bases which are found in plants. Arginine (I), lysine (II), and, to a lesser extent, histidine (III), are well known in extracts of plant material. Methods for



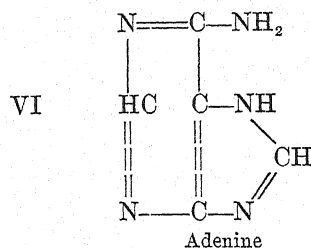
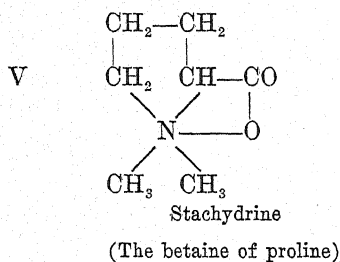
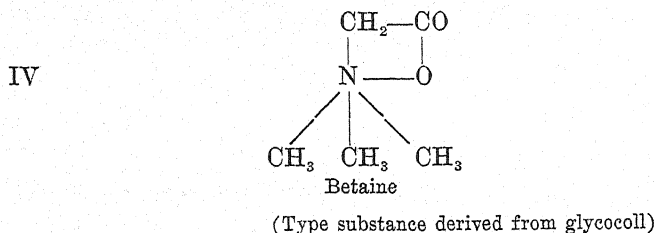
the approximately quantitative isolation of these three substances from the products of hydrolysis of proteins have been developed, and the general prin-

* The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

¹ Prepared on invitation from the Committee on Methods of Chemical Analysis, American Society of Plant Physiologists.

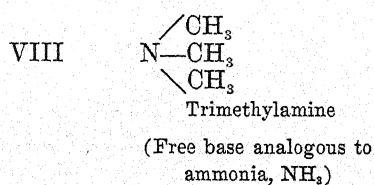
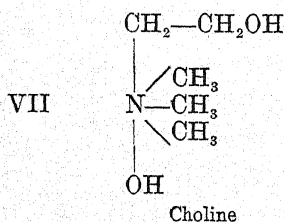
ciples underlying these methods are applicable to the study of plant extracts.

The betaines (IV, V) are formally related to the protein amino-acids since they may be regarded as methylation products of these substances.



They occur widely in plants and may form no inconsiderable part of the total basic nitrogen of the extract. Unfortunately we have no clean cut methods for dealing with the individuals of this group. To be sure there is a general precipitant, mercuric chloride in acid solution, but the separation of the individual betaines that may be found in one extract is largely a matter of fractional crystallization of suitable salts.

Beside those bases which may be regarded as related to the proteins, there are at least two other distinct types of substances which are usually encountered in plant extracts. These are, on the one hand, the weakly basic purines (VI) and, on the other, relatively strong bases such as choline (VII) and trimethylamine (VIII). Purines are intimately associated with



nucleic acid which is supposed to be present in cell nuclei, while choline is known to be associated with the phosphorized fats, and may possess other, as yet unsuspected, affiliations.

In the present discussion no account will be taken of that complex and economically important group of plant bases known as alkaloids. These do not seem to be nearly so widely distributed as the simpler bases and, moreover, alkaloid chemistry possesses a technique of its own which differs from that required in the study of the simple bases.

It is necessary to appreciate that these types of substances, which have been mentioned as probable components of any particular plant extract, by no means exhaust the possibilities. We know of their presence in plants simply because they, for the most part, possess properties which render isolation and identification easy. But there is every reason to expect that further study may reveal the presence of still other basic substances which, while they may be known as chemical possibilities or as specimens in the museum of the organic chemist, have not yet been shown to occur in nature.

The problem of the investigation of the bases which occur in plant extracts may, for the present purpose, be reduced to that of the choice of suitable reagents. This choice is severely circumscribed by two general requirements: first, the reagent must not introduce nitrogen and, second, it must be readily and completely removable by some manipulation which does not introduce conditions that might be expected to alter the other organic substances in the solution. These requirements cannot always be met; in fact it is seldom that we can remove reagents and, at the same time feel confident that other components of the solution have undergone no change. We have, therefore, a condition in which judgment and experience with each individual plant extract must play a dominant part. General prescriptions are, at the present time, of doubtful validity and may prove highly mischievous.

Phosphotungstic acid has been employed for many years as a general precipitant for bases and, when dealing with the mixture of amino-acids that is obtained from the hydrolysis of proteins, it is fairly definite in its action. With plant extracts, however, many circumstances combine to render a determination of the nitrogen precipitated by this reagent an uncertain measure of the true basic nitrogen.

To illustrate this statement, experiments² carried out with extracts from the alfalfa plant and from yeast may be described. The alfalfa extract was prepared by moistening the ground material (leaf and stem) with ether and pressing out in the hydraulic press. The juice was rapidly heated to 81° C.

² These experiments have been in part more fully described in papers in the Jour. Biol. Chem. See bibliography.

to destroy enzymes and coagulate proteins, filtered and concentrated, and alcohol was then added to make a final concentration of 50 per cent. by volume. The precipitate so produced was removed and the filtrate concentrated. This solution contained no detectable quantity of proteins, and is referred to in the following as "alfalfa filtrate." In order to remove phosphates and also those organic acids which form insoluble barium salts, an excess of barium hydroxide was added and the precipitate removed. An equal volume of alcohol was then added to the filtrate and a second precipitate removed. The first precipitate contained phosphoric and citric acids but little nitrogen, while the second contained malic and malonic acids³ together with nitrogenous compounds accounting for nearly 14 per cent. of the total nitrogen of the protein free extract. The filtrate from these precipitates was freed from reagents and treated with mercuric acetate, sodium carbonate and alcohol (NEUBERG's reagent) (1, 3, 4), a procedure which precipitates amino-acids, purines, and bases containing an amino group very completely, but which does not precipitate the methylated bases. This precipitate was decomposed with hydrogen sulphide and the solution was assumed to contain the greater part of those amino-acids and bases, exclusive of betaines and other methylated bases, which occur in the juice of the alfalfa plant. In order to separate the basic substances, phosphotungstic acid was then added in the usual way, and the precipitate decomposed by grinding with barium hydroxide five times, first in the cold and finally at boiling temperature. The fifth extract appeared to contain little, if any, organic material.

The filtrate from the phosphotungstic acid precipitate was treated with an excess of barium hydroxide to remove phosphotungstic and sulphuric acids and the precipitate thoroughly washed. Finally the excess of barium hydroxide was exactly removed from both fractions by means of sulphuric acid. This is the customary procedure for the separation of bases from non-basic substances. The data which were obtained are given in table I.

In view of these results what is one to take as the indicated basic nitrogen? Is it the nitrogen of the base fraction, amounting to 19.6 per cent. of the original nitrogen of the alfalfa filtrate, or should the nitrogen which could not be removed from the barium phosphotungstate and sulphate precipitate, amounting to 4.56 per cent. more of the original nitrogen, be added?

A still more ambiguous case was encountered in an examination of an extract from yeast. The precipitate obtained with mercuric acetate and sodium carbonate was divided into two parts, one of which was soluble in 5 per cent. sulphuric acid, and the other insoluble. This part contained 33.4

³ Unpublished data. For the presence of these hydroxy-acids in alfalfa, see citation (2).

TABLE I

THE DISTRIBUTION OF NITROGEN OBTAINED BY MEANS OF PHOSPHOTUNGSTIC ACID IN A FRACTION FROM ALFALFA FILTRATE

FRACTION	WEIGHT	PROPORTION OF NITROGEN OF ALFALFA FILTRATE
	gm.	Per cent.
Total nitrogen of solution.....	28.60	51.9
Ammonia nitrogen of solution.....	1.54
Total nitrogen of base fraction.....	10.81	19.6
Ammonia nitrogen of base fraction.....	0.32
Nitrogen in Ba phosphotungstate and BaSO ₄ (base fraction)	2.57	4.56
Total nitrogen of filtrate from bases.....	12.46	22.6
Ammonia nitrogen of filtrate from bases.....	0.24
Nitrogen in Ba phosphotungstate and BaSO ₄ (filtrate from bases).....	1.40
Total nitrogen recovered.....	23.27	42.19
Nitrogen lost in Ba phosphotungstate and BaSO ₄	3.97	7.1
Ammonia nitrogen lost*.....	0.98
Nitrogen unaccounted for.....	0.38

* This ammonia was lost from the alkaline solutions employed in the removal of phosphotungstic acid as its barium salt.

gm. of nitrogen, of which 13.84 gm. were obtained in the base fraction, while no less than 6.65 gm. were retained by the barium phosphotungstate and sulphate. In this case it was a question whether one should regard 13.84 gm. as the basic nitrogen of this fraction, or 20.49 gm., *i.e.*, 21.5, or 31.8 per cent. of the original nitrogen of the yeast extract.

A partial explanation of the difficulty was obtained by an examination of the barium phosphotungstate precipitate. It was boiled with concentrated hydrochloric acid for an hour, diluted and filtered. This extracted nearly one-quarter of the nitrogen. Further treatment with acid, finally boiling for 24 hours with 20 per cent. hydrochloric acid, extracted nearly another quarter of the nitrogen. The hot extracts on cooling deposited barium chloride, and, as they contained much free phosphotungstic acid, the small amount of organic bases likewise present separated as phosphotungstates. These were separated from the barium chloride by extraction with acetone. In all somewhat over one gram of nitrogen which appeared to belong to basic substances, and over two grams of nitrogen which appeared to belong to simple mono-amino-acids, were obtained. The basic substances were not further investigated, but the mono-amino-acid fraction yielded

appreciable quantities of glutaminic and aspartic acid while, in addition, a mono-amino mono-carboxylic acid fraction was obtained which, on evaporation, deposited crystals resembling leucine and valine. The occurrence of a positive Millon's reaction indicated the presence of tyrosine, while the fact that the amino nitrogen amounted to only 63 per cent. of the total nitrogen, in spite of the severe hydrolysis to which the solution had been subjected, indicated that proline might also be present.

To sum up, this experiment shows that, in addition to basic substances, phosphotungstic acid precipitated compounds, probably polypeptides, which yield simple mono-amino-acids on hydrolysis. It is, of course, impossible to determine from the present evidence whether the mono-amino-acids were associated with bases as basic polypeptides or whether we were dealing with polypeptides containing only mono-amino-acids. The fact remains that by no means all of the nitrogen precipitated by phosphotungstic acid belonged to strictly basic substances.

Examination of the solution of, presumably, basic substances obtained from alfalfa filtrate by decomposition of the phosphotungstates with barium hydroxide has also given evidence of the presence of nitrogen in substances which are not bases. The standard procedure with such a solution is to obtain fractions representing silver compounds insoluble in acid (the purine fraction), silver compounds insoluble in alkali (the histidine and arginine fraction), and a filtrate (the lysine fraction). Further work is necessary before these fractions can be separated from each other with certainty, and still more before we can isolate the individual substance quantitatively from each fraction. However, it may be illuminating to describe the results obtained from the lysine fraction, as they illustrate the difficulties encountered in this type of work. This solution contained four grams of nitrogen or 7.4 per cent. of the nitrogen of the alfalfa filtrate. It was treated with an excess of mercuric chloride and neutralized to litmus. The resulting precipitate contained 2.6 gm. of nitrogen. The filtrate was made alkaline and a second precipitate removed which contained 1.0 gm. of nitrogen. From the first precipitate less than 0.1 gm. of nitrogen was obtained as lysine by crystallization of the picrate. The mother liquors were freed from picric acid and a determination of total and amino nitrogen indicated that 38.9 per cent. of the nitrogen occurred as α -amino groups. The material was subjected to hydrolysis with acid after which it was found that 71 per cent. of the nitrogen occurred in α -amino groups. The solution was next treated with phosphotungstic acid, the precipitate decomposed, and nearly one-half a gram of nitrogen was then recovered as lysine picrate. The filtrate from the lysine phosphotungstate, when freed from reagents, yielded tyrosine and a mixture of leucine and valine with other amino-acids.

A similar type of material was found in the alkaline mercury precipitate. No lysine could be obtained from this solution at all until it had been subjected to hydrolysis. The hydrolysis raised the proportion of α -amino nitrogen from 51 to 81 per cent. and small amounts of lysine could then be readily obtained by crystallization of the picrate. Tyrosine and other mono-amino-acids were present in the mother liquors.

It is evident, therefore, that the "lysine fraction" obtained by the usual procedure contained much nitrogen in forms other than lysine, and it is highly probable that we were here dealing with a mixture of polypeptides in some of which lysine was a constituent.

It would be tedious to continue the description of the behavior of these different basic fractions. The purine fraction consisted essentially of purines with little extraneous material, but in the arginine fraction, as in the lysine fraction, there were peptides, and some of these may have contained arginine as a constituent.

Taken together, therefore, it seems clear that we are not yet in a position to assume that the nitrogen precipitated by phosphotungstic acid from plant extracts is wholly basic in nature, and it would not be going too far to state that this reagent is far less selective than it is sometimes thought to be. This must not be taken to mean that we should discontinue its use; it simply means that the results obtained with phosphotungstic acid must be regarded as furnishing outside limits to the true basic nitrogen. Further examination of the material precipitated by it is necessary.

In this laboratory extensive use has been made of a procedure devised by NEUBERG and KERB (1) for the precipitation of certain basic substances as well as amino-acids. Mercuric acetate and sodium carbonate are added alternately in such a way as to maintain an alkaline reaction until further addition of either solution produces a yellow precipitate. At this point an equal volume of alcohol is added to promote flocculation of the precipitate. To illustrate the effectiveness of this procedure as a means of precipitating amino nitrogen the results of an examination of a hot-water extract from yeast may be quoted. This extract contained 57.2 gm. of total, and 23.4 gm. of amino nitrogen. The filtrate from the mercuric acetate and sodium carbonate precipitate contained 5.2 gm. of total and only 0.67 gm. of amino nitrogen. As the volume of the filtrate measured over 26 liters, the small amount of amino nitrogen which escaped precipitation probably represents the solubility of the mercury compounds.

The advantage of this procedure is that it affords a sharp separation of bases containing methylated amino groups, such as choline and the betaines (IV, V, VII, and VIII) from other basic substances and from amino-acids. In the examination of alfalfa filtrate a solution containing 41.1 gm. of total and 10.0 gm. of amino nitrogen was treated in this way. The precipitate

contained 28.6 gm. of total and 10.1 gm. of amino nitrogen, while the filtrate contained 7.89 gm. of total and only 0.31 gm. of amino nitrogen. The slight discrepancy in the quantities of amino nitrogen recovered is probably due to experimental error, while the loss of 4.6 gm. of nitrogen is mainly due to the 3.38 gm. which could not be recovered from the large amount of mercuric sulphide obtained when decomposing the mercury precipitate. Of the 7.89 gm. of nitrogen in this filtrate, 4.67 gm. belonged to substances precipitable by phosphotungstic acid and 3.68 gm. of this nitrogen were obtained as crystalline derivatives of stachydrine (V) and choline (VIII) (4), while traces of betaine (IV) and trimethylamine (VIII) were also present. Taking into consideration the difficulty of isolating crystalline derivatives in quantitative yield it is apparent that this basic fraction contained little except bases of the methylated type.

A similar fraction from yeast extract consisted almost entirely of choline and nicotinic acid (5).

The results with these fractions are in marked contrast to those obtained when phosphotungstic acid precipitates are produced in solutions containing the amino-acids and peptides present in the original extract. The nitrogen precipitated by phosphotungstic acid from these less complex solutions appears in fact to consist entirely of basic nitrogen. The conclusion to be drawn is that it would be advisable to accept the total nitrogen precipitated by phosphotungstic acid as a measure of the basic nitrogen only when this precipitate has been produced in a relatively simple mixture. Precipitation with this reagent can be regarded, in general, only as a preliminary step.

Our present information indicates that extracts obtained from plants are usually extremely complex mixtures. Consequently their chemical examination cannot fail to be difficult and laborious. Too much account cannot, as yet, be taken of the results of indirect methods of analysis. When a substance has once been shown to be present we are, to some extent at least, justified in using a short-cut analytical method for its estimation. But in the lack of such information, the interpretation of the results of these methods becomes a matter of speculation and uncertainty. This may sound discouraging to plant physiologists but it must be remembered that we have well nigh passed through the era of simplification in science. The older beautifully simple statements of the physical laws of nature are already undergoing a process of complication. Second and third terms are being added to our equations to care for the slight discrepancies which are found when more accurate methods of measurement are employed. Animal physiology is becoming a science in which a broad grounding in physical chemistry is increasingly necessary and it is certain that plant physiology is des-

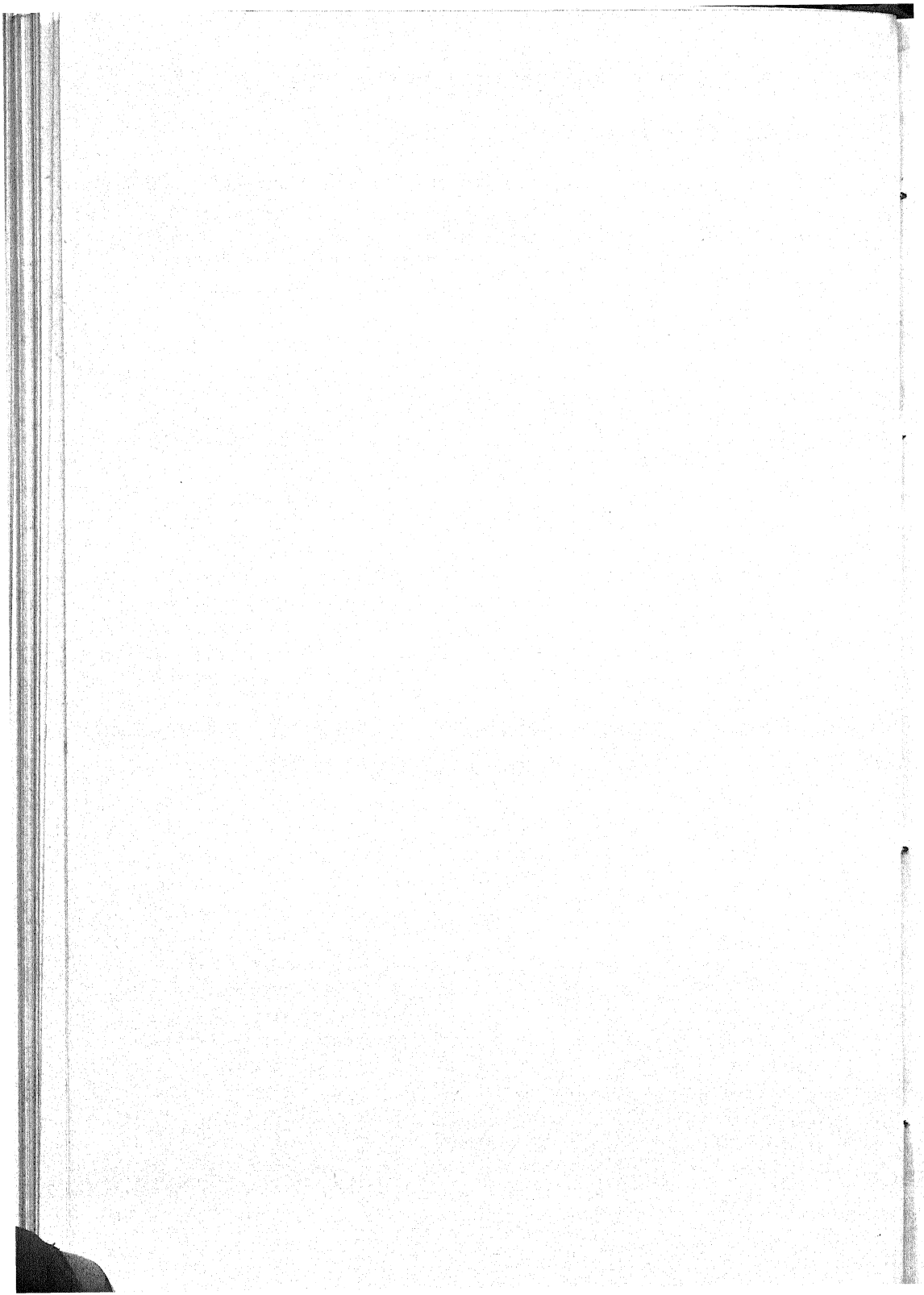
tined to become a field in which organic chemistry will play an even more significant part than it does at present.

The facts that lysine occurs in this plant, and choline in that, are interesting, but not as yet important. What is important, however, is to get a broad insight into the chemical environment in which the process of life is going on. The chemically trained physiologist and the physiologically trained chemist will then, perhaps, be able to attain to a clearer conception of what this process may be.

LABORATORY OF THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION,
NEW HAVEN.

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ELECTRODIALYSIS AS A MEANS OF STUDYING BIOCHEMICAL DIFFERENCES IN ABNORMAL APPLE TISSUE*

JAMES C. MOORE, R. G. REEVES, AND R. M. HIXON

(WITH SIX FIGURES)

Within recent years electrodialysis has come into favor as a means of studying certain chemical and physical phenomena. This method has been employed by DHÉRE and GORGELEWSKI (4), ADOLF and PAULI (1), FREUNDLICH and LOEB (6), BERNHARD and BEAVER (2), and ETTISCH and BECK (5) as a means of determining the composition of serums; by LISBONNE and VIELQUIN (10), and FRICKE *et al.* (7) in their investigations of enzyme activity. TAYLOR, BROWN, and SCOTT (15) used electrodialysis for separating the active and inactive constituents of insulin; HOFFMAN and GORTNER (8) found it a suitable means for splitting free agar acid from its calcium salt. KÖNIG (9), MATTSON (11), and CLARK *et al.* (3) have studied the replaceable bases of soils by the same method.

From the work of CLARK just mentioned on the electrodialysis of soils, the idea of testing this method for the study of biochemical phenomena of plant tissues was conceived, and as a review of the literature at hand revealed no report of any such investigations, the present study was undertaken.

Description of electrodialysis cell

MATTSON (11) has described a three-chambered cell which he used for the study of replaceable bases in soils. CLARK, HUMFELDT and ALBEN (3) modified the MATTSON cell by putting in a cooling system made of ordinary glass tubing. The diagrams in figs. 1 and 2 show the essential features of such a cell. A¹ and A² in fig. 1 are made of hard rubber, $\frac{3}{8} \times 8 \times 6$ inches, with an outlet at the bottom for draining the cell. B, C, and D are made of $\frac{3}{4}$ -inch soft rubber as shown in fig. 2. E¹ and E² are parchment paper membranes. The entire cell is clamped together by means of six bolts. The above cell warped badly due to the strain of the bolts when tightened. To overcome this and to allow for greater space for biological material, the following modifications were made: (1) the cell was made of soft rubber throughout; (2) the $\frac{3}{8}$ -inch soft rubber ends were reinforced with $\frac{1}{8}$ -inch

* Contribution from the Pomology Section and the Department of Chemistry, Iowa State College.

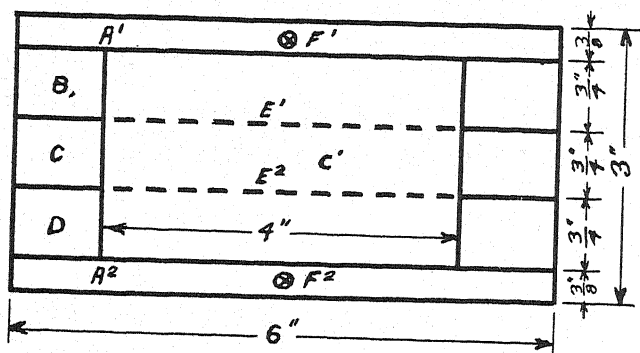


FIG. 1. Three chambered electro-dialysis cell. Top view.

brass plates; (3) the cell was made nine inches high, giving a space of $4 \times 8 \times \frac{3}{4}$ inches for the material under investigation. This compartment will readily hold 200 grams of apple parings (wet weight) and the other two compartments about 300 milliliters of water. F^1 and F^2 are ordinary binding posts for connecting the electrodes and lead wires. The electrodes, described by CLARK *et al.* (*loc. cit.*) were used. They consisted of a platinum wire gauze for the negative cell and a three wire electrode for the positive cell.

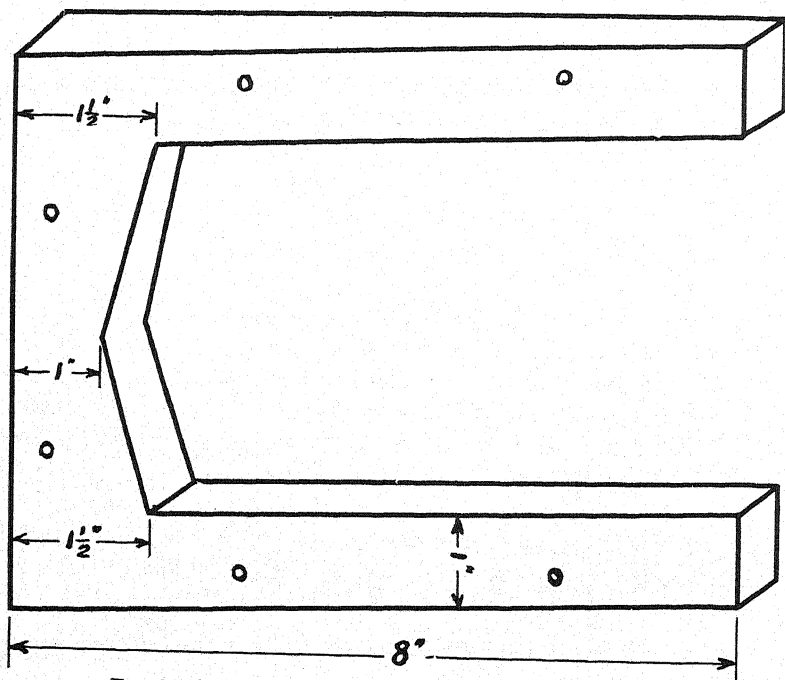


FIG. 2. Section of cell showing U-shaped central portions.

Materials and methods

The materials used for this investigation were normal and abnormal Jonathan apples.

The normal apples were free from surface blemishes, of good size and color, complying with the requirements for A grade fruits. Abnormal apples were of the same general size, color and condition of flesh, but were badly affected with a superficial dark brown or black circular spot commonly known as Jonathan spot. In most cases about 50 to 75 per cent. of the total area of the apples was affected; according to the arbitrary classification used by PLAGGE and MANEY (13) apples of this type would fall into the bad class.

These apples had been in storage from October, 1926, to June, 1927. They were from the same orchard and so far as known were handled in a similar manner throughout the period of storage.

The fruit was peeled with a small hand-operated apple parer, the cutting blade being adjusted to peel as thin as possible, which gave a peel about 1 to 2 millimeters in thickness. The parings were immediately weighed on a trip balance and then placed in compartment C^1 of the cell described in fig. 1. Two hundred milliliters of distilled water were then poured into compartments A and B, and the peels in C^1 were covered with distilled water.

A direct current of 110 volts was used for all samples. The amperage was recorded at the end of each time interval, which was taken from the closing of the circuit with the addition of water to the cell, to the breaking of circuit by removing the dialysate from the compartments. The time required to drain and refill these compartments varied from two to three minutes. A twenty-minute period, therefore, actually would be about seventeen to eighteen minutes. The dialysate was drained into 300 ml. Erlenmeyer flasks, which were tightly closed with rubber stoppers until they could be titrated. The material which came out on the basic side was titrated electrometrically,² using a student potentiometer, with 0.1 N HCl which had been standardized against AgNO_3 .

The solution from the cathode cell was titrated with 0.1 N NaOH using phenolphthalein as an indicator. Preliminary runs were made using varying amounts of tissue and various time intervals for changing the dialysate. The final method adopted for the experiment follows: The cell was thoroughly cleaned, new parchment membranes put in place and then dialyzed for a period of one hour with frequent changes in order to remove inorganic

² The pigment of the Jonathan apple migrates to the negative cell; this gives a solution with sufficient color to mask an indicator. Further work is going forward on this phenomenon.

material from the paper. A weighed quantity (in each case 184.5 gm.) of wet apple parings was placed in the cell and the current turned on. The time intervals used were two twenty-minute periods, one thirty minutes, two one-hour, one two-hour, two four-hour, and one twelve-hour periods. This makes a total of twenty-five hours and ten minutes elapsed time.

These intervals for changing dialysate proved very satisfactory for keeping the temperature of the middle compartment below 55° C.; a temperature above this was considered to be injurious to the tissue. In only two cases did the temperature go above 45° C., both of these being in series "3a."

After titration the dialysates were grouped according to the initial E.M.F. and evaporated to dryness on a sand-bath and ashed for the purpose of qualitative analyses.

The series were indicated as normal and abnormal; 1 N, 2 N, 3 N, and 4 N being the four different samples of normal apple parings, and 1 A, 2 A, 3 A, and 4 A being the four different samples of abnormal tissue. As previously mentioned 1 N, 2 N, 1 A and 2 A were run in different ways and will not be used except for comparison of methods. 3 N, 4 N, 3 A and 4 A were run identically and consisted of equal quantities of tissue. Therefore, these samples are comparable.

Results

Table I gives the time interval of changing dialysate, the ml. of normal acid, and the ml. of normal base of dialysate as calculated from titration of the dialysate at the end of each period.

TABLE I

TIME OF CHANGING DIALYSATE AND MILLILITERS OF ACID OR BASIC CONSTITUENT AT END OF EACH PERIOD

1 N			2 N		
N ACID	TIME	N BASE	N ACID	TIME	N BASE
ml.		ml.	ml.		ml.
1.725	1 hour	5.511	0.334	15 minutes	1.234
0.799	1 hour	1.073	0.826	45 minutes	2.179
0.431	1 hour		0.684	1 hour	0.254
0.212	1 hour		0.317	1 hour	
0.200	1 hour		0.216	1 hour	
0.211	1 hour		0.220	1 hour	
0.183	1 hour		0.312	2 hours	
0.148	1 hour		0.275	2 hours	
0.174	1 hour		0.321	3 hours	
0.138	1 hour		0.50	4 hours	
0.128	1 hour		0.780	8 hours	
0.156	1 hour		0.50	12 hours	
2.065	12 hours 40 minutes		1.00	4 hours 5 minutes	
6.570		6.584	5.485		3.667

Table I indicates that practically all of the basic constituents were removed from 1 N during the first hour, likewise they were removed from sample 2 N, in which case the time interval was different and also a different weight of tissue. In case of sample 1 N the ml. of normal acid are practically equivalent to the basic constituents. On the other hand in 2 N the normal acid is considerably higher than normal base.

Table II gives the physical and chemical measurements of the above samples. The amperage and temperature were taken at the end of each time interval. The E.M.F. was determined by the student potentiometer. The pH was taken from tables compiled by SCHMIDT and HOAGLAND (14).

TABLE II

PHYSICAL AND CHEMICAL MEASUREMENTS OF SAMPLES 1 N AND 2 N

AMPERAGE		INITIAL E. M. F.		PH		TIME		TEMPERATURE	
1 N	2 N	1 N	2 N	1 N	2 N	1 N	2 N	1 N	2 N
						hours	hours	°C.	°C.
0.89	0.5	0.97	0.918	11.63	10.72	1.0	0.25	47	30
0.41	0.5	0.857	0.925	9.68	10.85	1.0	0.75	30	37
0.20	0.31	0.475	0.80	3.2	8.74	1.0	1.0	22	26
0.09	0.1	0.559	0.500	4.6	3.66	1.0	1.0	19	21
0.06	0.05	0.545	0.475	4.4	3.24	1.0	1.0	21	19
0.02	0.04	0.534	0.575	4.2	4.93	1.0	1.0	20	20
0.01	0.05	0.496	0.55	3.5	4.51	1.0	2.0	21	19
0.06	0.04	0.502	0.52	3.6	4.0	1.0	2.0	21.2	18
0.04	0.04	0.505	0.485	3.7	3.41	1.0	3.0	21	19

Table II shows that the time interval has considerable effect upon the conductivity of the solution as shown by the amperage. 2 N in the first two periods has almost one-half the amperage of 1 N, but in each of the other periods the amperage is higher. Sample 1 N weighed 163 gm. while sample 2 N weighed 123 gm.

Tables III and IV show comparisons for similar data on abnormal tissue, the wet weight of tissue being the same in each sample. However, in 1 A, the time interval was varied slightly and the electrode was the same as in the other runs, i.e., platinum wire gauze and one trident-shaped three-wire platinum electrode. In 2 A both electrodes were trident.

Table III indicates that the size of the electrode has considerable influence on the rate of dialysis, as is indicated by the amperage and quantity of titratable acids and bases which were removed from the tissue. Table IV gives the physical measurements of the same samples.

The amperage of 2 A as shown in table IV goes through a gradual increase to a maximum of 0.6 at the end of two hours and ten minutes, then

TABLE III
MILLILITERS OF ACID AND BASE IN DIALYSATE AT END OF EACH PERIOD

1 A			2 A		
TIME	ACID	BASE	TIME	ACID	BASE
Minutes	ml.	ml.	Minutes	ml.	ml.
20	0.587	3.199	20	0.376	1.153
20	0.413	1.914	20	0.174	0.265
20	0.358	1.233	30	0.293	0.945
30	0.661	1.948	60	1.084	2.894
30	0.514	0.922	60	1.046	1.464
60	0.973	0.980	120	1.358	0.922
60	*	0.034	240	1.927	
120	*	0.000	240	1.239	
240	1.322	0.000	720	2.193	
240	1.001	0.000			
720	2.130				

* Sample lost.

TABLE IV
PHYSICAL MEASUREMENTS OF ABNORMAL SERIES 1 A AND 2 A

AMPERAGE		INITIAL E.M.F.		PH		TIME		TEMPERATURE	
1 A	2 A	1 A	2 A	1 A	2 A	1 A	2 A	1 A	2 A
						Minutes	Minutes	°C.	°C.
0.85	0.4	0.987	0.945	11.9	11.19	20	20	35	26
0.55	0.25	0.974	0.913	11.67	10.65	20	20	33	23
0.49	0.30	0.956	0.919	11.37	10.75	20	30	29	23
0.56	0.60	0.970	0.970	11.60	11.60	30	60	30	23
0.41	0.5	0.930	0.939	10.93	11.09	30	60	26	31
0.45	0.45	0.918	0.928	10.72	10.89	60	120	31	30
0.36	0.44	0.711	0.587	7.23	5.13	60	240	24	27
0.18	0.15	0.584	0.621	5.08	5.71	120	240	22	22
0.13	0.18	0.540	0.598	4.34	5.32	240	720	23	22
0.10	0.12	0.545		4.42		240	1020	21	23
0.20		0.615		5.6		720		21	

gradually drops to 0.12 at the end of 25 hours and 10 minutes. When a platinum gauze is used for the negative electrode the amperage begins at a maximum and drops rapidly to the end of the fourth hour, after which there is a gradual decrease.

Table V shows the milliliters of normal acid and base of the dialysate from normal apple peels at the end of each period, and the total amount removed. Both samples were identical and were treated in the same manner; therefore they serve as checks.

TABLE V

MILLILITERS OF ACID AND BASE IN 184.5 GRAMS OF NORMAL APPLE PEELS

TIME	3 N		4 N	
	ACID	BASE	ACID	BASE
Minutes	ml.	ml.	ml.	ml.
20	0.688	3.678	0.523	2.44
20	0.376	1.616	0.303	1.568
30	0.597	1.591	0.431	1.648
60	0.991	1.095	1.092	1.360
60	0.881	0.075	0.551	0.104
120	0.927		0.798	
240	1.386		0.918	
240	0.707		0.706	
720	1.377		1.450	
Total 1510	7.930	8.055	6.772	7.120

While there are variations in the above checks the difference is very insignificant as compared to the large differences between normal and abnormal tissue. Table VI shows the physical measurements of the same tissue.

TABLE VI

PHYSICAL MEASUREMENTS OF NORMAL TISSUE

AMPERAGE		INITIAL E.M.F.		PH		TIME	TEMPERATURE	
3 N	4 N	3 N	4 N	3 N	4 N	3 N AND 4 N	3 N	4 N
						Minutes	°C.	°C.
0.95	0.75	0.983	0.983	11.83	11.83	20	40	32
0.65	0.52	0.960	0.974	11.44	11.61	20	35	34
0.55	0.51	0.959	0.973	11.42	11.66	30	35	34
0.40	0.52	0.920	0.949	10.75	11.25	60	35	38
0.30	0.28	0.80	0.836	8.7	9.34	60	30	26
0.24	0.20	0.6	0.512	5.4	3.85	120	30	25
0.21	0.2	0.58	0.568	5.1	4.8	240	31	24
0.07	0.08	0.545	0.525	4.4	4.0	240	20	21
0.05	0.06	0.60	0.595	5.3	5.27	720	23	21

With a few exceptions the above runs check each other rather closely as will be brought out in figure 4.

Table VII and table VIII are the same as the two preceding tables except that they are taken from data on the abnormal series.

TABLE VII
MILLILITERS OF ACID AND BASE IN ABNORMAL TISSUE

TIME	3 A		4 A	
	ACID	BASE	ACID	BASE
Minutes	ml.	ml.	ml.	ml.
20	1.054	5.286	1.084	5.476
20	0.886	3.661	0.927	4.07
30	0.763	1.735	0.991	1.85
60	1.303	2.375	1.119	1.314
60	0.716	0.069	0.734	
120	1.028		0.835	
240	1.519		1.00	
240	1.060		0.954	
720	2.611		2.395	
Total 1510	10.940	13.126	10.039	12.710

The above data indicate that there is a deficiency of acid constituents in the abnormal apple peels.

The physical measurements of the abnormal series 3 A and 4 A are presented in table VIII.

TABLE VIII
PHYSICAL MEASUREMENTS OF ABNORMAL APPLE PEELS

AMPERAGE		INITIAL E.M.F.		PH		TIME	TEMPERATURE	
3 A	4 A	3 A	4 A	3 A	4 A	3 A AND 4 A	3 A	4 A
						Minutes	° C.	° C.
1.60	1.65	0.998	0.995	11.91	12.03	20	45	52
1.0	1.05	0.977	0.986	11.73	11.88	20	40	54
0.8	0.7	0.946	0.954	11.20	11.34	30	33	40
0.6	0.51	0.906	0.894	10.52	10.32	60	32	34.5
0.25	0.25	0.709	0.643	7.2	6.08	60	22	28
0.2	0.18	0.626	0.600	5.7	5.35	120	28	19
0.14	0.09	0.585	0.646	5.1	6.13	240	19	20
0.08	0.1	0.549	0.584	4.49	5.08	240	19	25
0.01	0.09	0.555	0.591	4.59	5.2	720	29	22

The data in tables V and VII are presented in graphical form in figure 3. This figure brings out the fact that abnormal apple peels have a significant difference in the dialysable chemical components as compared with normal apple peels. The data in tables VI and VIII are plotted in a similar

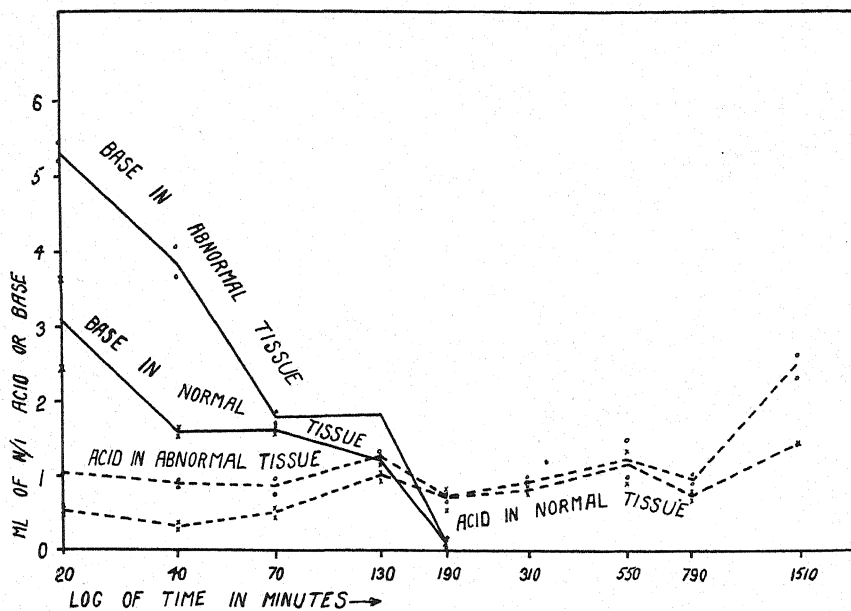


FIG. 3. Milliliters of N/1 acid or base in normal and abnormal apple tissue.

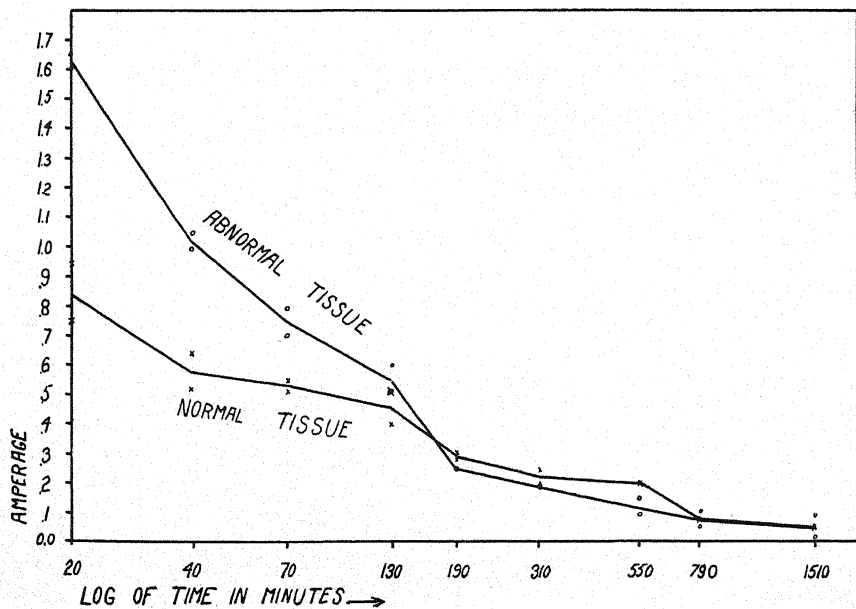


FIG. 4. Amperage plotted against time—normal and abnormal tissue.

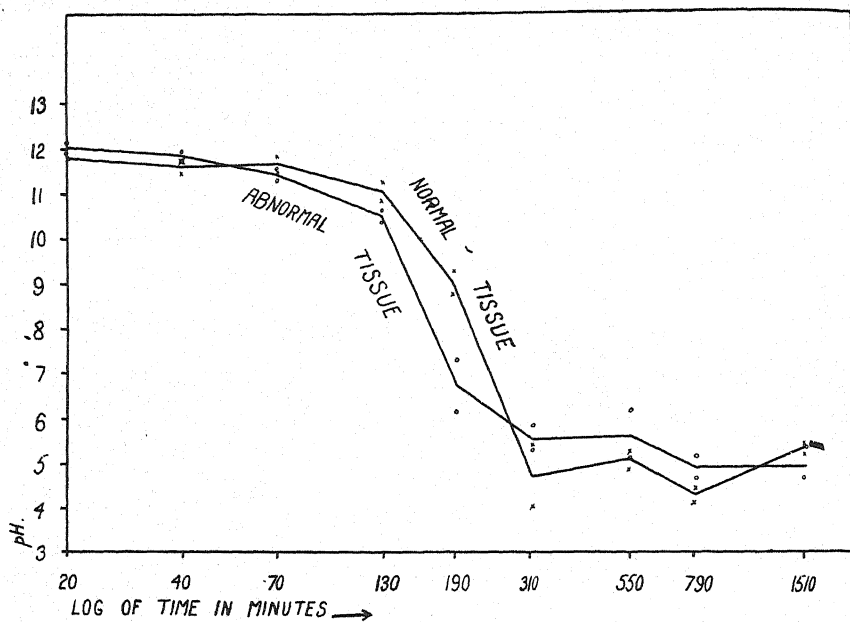


FIG. 5. Initial pH of dialysate at each time interval.

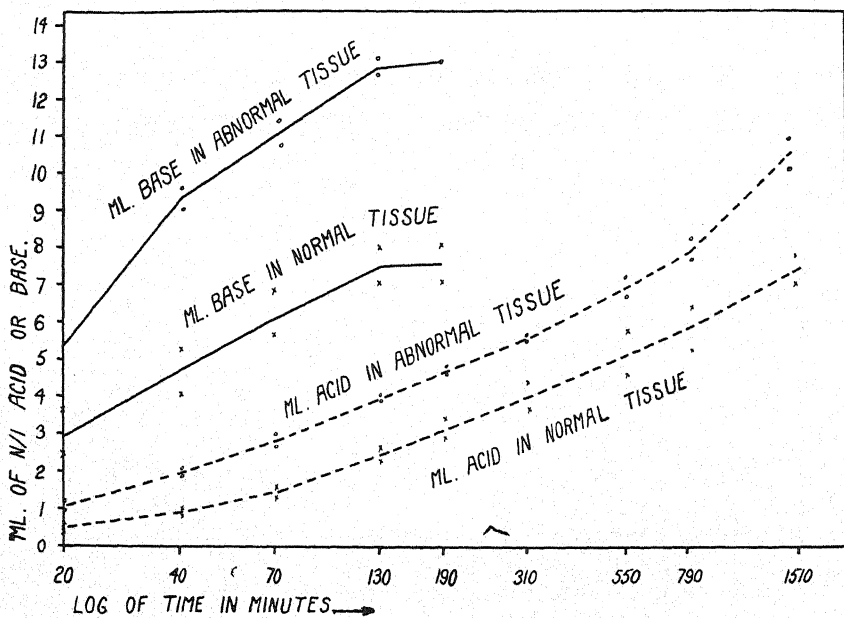


FIG. 6. Total milliliters of acid and base accumulative in elapsed time.

way in figure 4, amperage being plotted arithmetically while time is plotted logarithmically.

It is interesting to note that the drop in amperage between 130 minutes and 190 minutes corresponds to the removal of the titratable bases as shown in figure 3.

Figure 5 shows the initial pH of the basic solution at the end of each period of time. It should be noted that the normal tissue reaches a pH of 7 after approximately 250 minutes of dialysis, while the abnormal tissue begins much higher and reaches the neutral point much sooner or in about 200 minutes.

Table IX summarizes the data given in tables V and VII. Milliliters of acid and base are indicated accumulatively against elapsed time.

TABLE IX
SUMMARY OF DATA IN TABLES V AND VII

TIME	3 N		4 N		3 A		4 A	
	ACID	BASE	ACID	BASE	ACID	BASE	ACID	BASE
Minutes	ml.	ml.	ml.	ml.	ml.	ml.	ml.	ml.
20	0.688	3.678	0.523	2.440	1.054	5.286	1.084	5.476
40	1.064	5.294	0.826	4.008	1.930	8.947	2.011	9.546
70	1.606	6.885	1.257	5.656	2.693	10.682	3.022	11.396
130	2.652	7.980	2.349	7.016	3.996	13.057	4.121	12.710
190	3.533	8.055	2.900	7.120	4.712	13.126	4.855	
310	4.460		3.698		5.740		5.690	
550	5.846		4.616		7.259		6.690	
790	6.553		5.322		8.319		7.644	
1510	7.930		6.772		10.930		10.039	

The above data are presented graphically in figure 6.

Discussion and summary

In connection with the cold storage investigation being carried on by the Pomology Section of this station, PENTZER (12) advanced the theory that Jonathan spot was caused by a loss of acids during storage. The data presented above apparently substantiates PENTZER's theory.

The data reported indicate that electrodialysis offers a convenient means of studying chemical differences in the non-colloidal constituents of normal and abnormal tissue. Quantitative differences which would be masked by large quantities of inert material are accentuated by this method of separating those portions which are soluble and chemically active.

Qualitative analyses on the dialysate indicate that potassium and sodium are removed from the tissue during the first hour. The calcium, magnesium, iron and aluminum are removed after 130 minutes elapsed time.

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GERMINATION AND GROWTH OF SEEDS AS DEPENDENT UPON SELECTIVE IRRADIATION*

GEORGE M. HIGGINS AND CHARLES SHEARD

(WITH TWO FIGURES)

The present study of the influence of selective irradiation on germination and growth of the cucumber seed was not undertaken to determine the cytoplasmic reactions to the component wave-lengths of light, but rather to secure further facts concerning the general response of protoplasm toward wave-lengths of known distribution, with especial emphasis on those responses incited by wave-lengths at or below the shorter end of the visible spectrum. If studies of this sort are to approach the proportions of a science, it is essential that the radiation employed throughout the period of experimental observation be of a known spectral distribution. Results that are attained without due regard for the character of the wave-length employed lend but slight value to the final analysis of the responses incited by any given component. Accordingly, the observations herein reported concern the results obtained in the germination and growth of cucumber seeds under filters of a known transmission, exposed to ultra-violet radiation for given periods of time. The source of the radiation employed was an air-cooled mercury-arc lamp¹ operated at 70 volts at a distance of 50 cm. The lamp was standardized or graded by the method of reaction of the normally unexposed skin of the upper arm to various periods of exposure to the radiation from the lamp at a distance of 45 cm. Three minutes' exposure was required to give a reaction of grade 2 (permanent erythema).

Throughout the experimental period of eight days, germination² and growth of the seeds were observed under the following conditions: Cucumber seeds were carefully selected and placed on moist blotters in the bottom of ten medium-sized flower pots. Two of these were covered with ultra-glass (Corning glass, 586 AW), which transmits wave-lengths from approximately 390 m μ to 320 m μ with a maximum of about 370 m μ (fig. 1), and carefully sealed with adhesive tape. For convenience these pots have been

* From the Division of Experimental Surgery and Pathology, and the Section on Physics, The Mayo Foundation, Rochester, Minnesota.

¹ The lamp used during these observations was made available through the courtesy of the Victor X-Ray Corporation.

² The term "germination" is here used to include the earlier growth up to the first appearance of the seedling.

designated Ia and Ib. Likewise, two of the pots were covered with a special vitaglass (made and marketed by F. E. Lamplough, M.A., Birmingham, England), which transmits all of the visible and the lesser wave-lengths down to 270 m μ . These pots have been designated IIa and IIb. Two other pots marked IIIa and IIIb were covered with ordinary window-glass, which

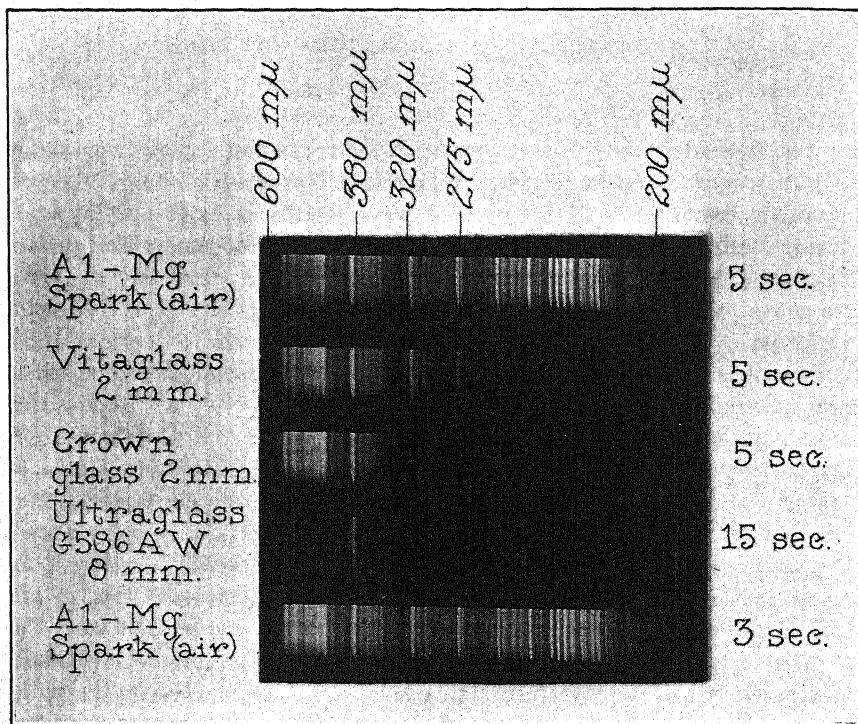


FIG. 1. Spectrograms showing the transmission of visible and ultra-violet radiation by vitaglass (Lamplough, England), ordinary window-glass, and ultra-glass (Corning, 586 AW).

transmits all the visible as well as the lesser wave-lengths down to 320 m μ . Pots IVa and IVb remained uncovered during the periods of irradiation, so that, in addition to the visible regions these seeds were exposed to wave-lengths possibly as low as 190 m μ . In the intervals between the periods of irradiation these pots were covered with ordinary window-glass to prevent evaporation. Pots Va and Vb were not exposed to radiation from the lamp but were covered with ordinary window-glass and served as controls throughout the experiment. Pots Ia, IIa, IIIa, IVa, and Va were kept constantly in a dark cabinet, except for the daily periods of irradiation, while

pots Ib, IIb, IIIb, IVb, and Vb were kept in ordinary daylight under window-glass. Pots Ia and Ib were exposed to the radiation of the quartz mercury-arc lamp for twenty minutes each day, while IIa, IIb, IIIa, IIIb, IVa, and IVb were exposed to the same radiation for five minutes daily. The prolongation of exposure under the ultra-glass was made necessary on account of the absorption of energy due to the greater thickness (see fig. 1). The temperature of the room and of the dark cabinet remained relatively constant during the entire time; that of the cabinet was slightly lower than that of the room. Drops of water were added to the various pots from time to time to maintain a relatively constant degree of humidity.

The experiments here described were repeated five times. Six to ten seeds were used in each container during each set of experiments. In the table and in the illustration (fig. 2) of length of roots under the filters used, we have given sample data which correctly portray the results which were found to occur under the experimental conditions cited in at least eighty per cent. of the cases. We have excluded the few seeds which, for one reason or another, proved to be non-germinating. The experiments were conducted during the months of July and August, 1926, and under conditions of temperature as uniform as possible. The average daily temperature was $75^{\circ}\text{C.} \pm 10^{\circ}$. Hence, in any completed series or group of experiments, all seeds were subjected to identical conditions with the one exception of the variation in the amount and character of the light received.

Observations

Considerable variation in the time of germination of the seeds in the various pots indicated very early that light rather than temperature was the differential factor involved. Observations and measurements of the seedlings were made at the end of the first forty-hour period and at the conclusion of each twenty-four-hour period for eight days. Measurements were all taken at the time of irradiation, so that no additional exposure to daylight was unnecessarily made for this procedure.

At the end of forty hours, with two twenty-minute exposures to the rays of the quartz mercury arc, all seeds in pots Ia and Ib had germinated and the average length of the new growths in each pot was 4.5 mm. Thus far no difference in the respective rates of growth was evident in the pot kept in the dark cabinet and that kept in daylight; but it was strikingly evident that the lesser wave-lengths of these initial exposures, transmitted by the ultra-glass, were sufficient to hasten the time and accelerate the rate of germination.

In pots IIa and IIb, covered with vitaglass which transmits the visible as well as the shorter wave-lengths down to 270 m μ , a difference in the rate

TABLE I

LENGTH IN MILLIMETERS OF SEEDLINGS GROWN UNDER VARYING EXPERIMENTAL CONDITIONS

ELAPSED TIME	I		II		III		IV		V	
	ULTRA-GLASS		VITA-GLASS		ORDINARY GLASS		DIRECT IRRADIATION		CONTROL	
	(A) DARK	(B) LIGHT	(A) DARK	(B) LIGHT	(A) DARK	(B) LIGHT	(A) DARK	(B) LIGHT	(A) DARK	(B) LIGHT
Hours	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
40	4	4	3	1	2	0	3	0	0	0
64	14	14	10	6	9	0	7	1	9	0
88	36	24	27	14	23	1	19	5	28	2
112	54	30	42	20	35	2	30	8	52	6
136	65	37	47	25	44	3	31	5	63	8
184	70	40	55	25	60	3	31	5	82	6

Pots I (A) and I (B) were exposed to the radiation of the mercury quartz lamp for twenty minutes each day. Pots II (A) to IV (B) inclusive were irradiated by the same source for five minutes daily.

Ultra-glass (Corning 586 AW) transmits from 390 to 320 $m\mu$ with a maximum at 370 $m\mu$. Vitaglass (Lamplough) transmits to 270 $m\mu$; ordinary glass to 320 $m\mu$.

of germination of the seeds was strikingly evident. Those seeds exposed to two five-minute periods of irradiation, and kept in the dark cabinet, had attained an average growth of 3 mm.; while those similarly exposed, but kept in the light, were but 1 mm. long. Here it would seem that the inhibitive effect evidenced in the growth of seeds in pot IIb may be due to the greater wave-lengths to which the seeds were constantly exposed. Although the vitaglass transmits more of the lesser wave-lengths than the ultra-glass, the shortness of the periods of exposure to the lamp, and thus to the visible light as well, was sufficient to counteract the added stimulative effect of the rays below 300 $m\mu$. Then, too, the lethal effect of lesser wave-lengths may have been sufficient to have induced coagulation and impeded normal germination.

A similar difference in the extent of the growth of the seedlings was evident in pots IIIa and IIIb. The average new growth of those seeds, covered by ordinary glass which transmits the visible and lesser wave-lengths down to 320 $m\mu$ and exposed to two five-minute periods of irradiation but kept otherwise in the dark, was 2 mm. On the other hand, those seeds similarly exposed, but kept constantly in the daylight under window glass, had not as yet shown signs of germination. This growth of 2 mm. of the seeds in pot IIIa does not equal that of the seeds grown under the ultra-glass, in which case there is an exclusion of all wave-lengths greater than 390 $m\mu$, with a

transmission of a limited region of radiant energy (390 $m\mu$ to 320 $m\mu$) in the near-ultraviolet region. In the case of the seeds in pot IIIb, where germination had not occurred, it is probably true that such stimulative action as may have been induced by wave-lengths as low as 320 $m\mu$ was in some way inhibited or counteracted by those of the visible spectrum to which the seeds were constantly exposed.

This interpretation is rendered only the more imperative by the evidence gained from seedlings grown in pots IVa and IVb. In these cases, the rays of the mercury-arc lamp were allowed to play directly on the seeds, differing thus from IIIa and IIIb in the absence of any filter. In the intervals between the direct exposures to the lamp, these pots were covered by ordinary glass and kept in their respective light and dark environments. The average growth of the seeds so exposed for two five-minute periods, and kept in the dark, was 3 mm.; while those kept in ordinary daylight showed no evidence of germination. Here, again, it would appear that the stimulative effect of the lesser wave-lengths of the lamp had incited a growth equal to that attained under the vitaglass when kept in the dark. On the other hand, such stimulative action as may have been induced by the periods of exposure was rendered ineffectual by the continued exposure to the greater wave-lengths of daylight. The difference in the growth of the seedlings attained under the vitaglass and the ordinary glass, when kept in daylight, may be explained on the basis that the vitaglass was constantly transmitting certain of the stimulative wave-lengths, while ordinary glass is impervious to those below 320 $m\mu$.

At the conclusion of this first forty-hour period, there was no evidence of germination of any of the seeds either in pots Va or Vb, to which the rays of the mercury-arc lamp had never been applied.

On the basis of these observations certain facts seem evident. The lesser wave-lengths are stimulative and accelerate the rate and time of germination, while the greater wave-lengths are inhibitive and seem to render ineffective the action of the violet and ultra-violet rays. Germination is either induced by the action of the light or by a temperature factor which is accessory to such experiments wherever an air-cooled lamp is employed. Subsequent growth of these seedlings must lead one to conclude that light-rays are in all probability the more inciting factors.

At the conclusion of the ensuing twenty-four-hour period, or sixty-four hours of experimental procedure, with three periods of exposure to the lamp's rays, further changes in the rate of germination and growth were evident. No appreciable difference in the rate of growth of the seedlings has yet occurred in those pots covered with the ultra-glass and kept in the dark and in the light. In pots Ia and Ib the average length of the seedling

was 14 mm., so that wave-lengths up to 400 $m\mu$ which are transmitted by the ultra-glass in daylight were not sufficiently inhibitive to counteract the stimulative effect of the ultra-violet rays during the early growth. In pots IIa and IIb, in which the seedlings were exposed to wave-lengths as low as 270 $m\mu$ for five minutes each day, the growth of those kept in the dark had exceeded by 60 per cent. that of the seedlings exposed to the greater wave-lengths of daylight. Likewise, those seedlings in pot IIIa, irradiated through window-glass which is impervious to rays shorter than 320 $m\mu$, had attained a growth only slightly less than that attained by the seedlings in IIa in which the wave-lengths as short as 270 $m\mu$ were effective. On the other hand, none of the seeds in pot IIIb as yet showed any signs of germination, a further evidence of the counteractive effect of the visible light, which had rendered ineffectual whatever energy had been received by the seeds during the periods of irradiation. Again, in pots IVa and IVb, the same differences in growth just considered for the seedlings in pots IIIa and IIIb obtained. The average growth attained in IVa was 7 mm. as opposed to an average growth of 1 mm. in IVb. The growth in IVb in contrast to the failure of germination of the seeds in IIIb is to be expected, since in the former case the seeds were directly exposed to irradiation, more of the shorter rays being thus made effectual, and a degree of energy secured that was not entirely offset in its effects by the longer rays of the visible to which the seeds were constantly exposed.

The end of this sixty-four-hour period showed most strikingly differences in the rates of germination and growth of the seedlings in pots Va and Vb, the normal controls. With no evidence of germination at the end of forty hours, growth of the seeds in the control pot in the dark had been so rapid as to produce a seedling at this time equal to those grown in pot IIIa, 9 mm. long. All the seeds in pot Vb, the normal control kept in the light, still failed to show signs of germination. The facility with which seeds germinate in the dark is a commonplace observation, and as common is the fact that daylight hinders germination. These observations would seem to point to the conclusion that the energy requisite for germination may be a derivative of the lesser wave-lengths, although no evidence is at present available as to what effect the infra-red may have. In the case of the seeds of pot Va, energy previously stored in the seed by solar irradiation was sufficient to induce germination when unhampered by visible light; but the added energy distributed to the seeds by the exposure to the radiation of the lamp served to increase the normal energy and to accentuate the time and rate of germination.

A greater difference in the germination and growth of the various seedlings was observed at the end of eighty-eight hours (fig. 2). During the last twenty-four hours seedlings grown under the ultra-glass, transmitting wave-

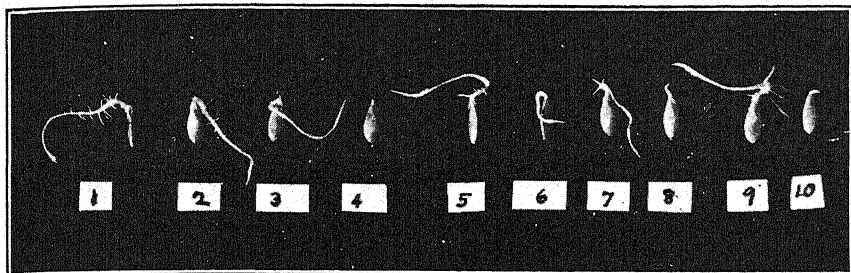


FIG. 2. The extent of growth of the cucumber seed after eighty-eight hours: (1) irradiation of the seed through ultra-glass, seed kept in the dark; (2) irradiation of seed through ultra-glass, seed kept in the light; (3) irradiation of the seed through ordinary glass, seed kept in the dark; (4) irradiation of the seed through ordinary glass, seed kept in the light; (5) irradiation of the seed through vitaglass, seed kept in the dark; (6) irradiation of the seed through vitaglass, seed kept in the light; (7) direct irradiation of the seed, otherwise kept in the dark; (8) direct irradiation of the seed, otherwise kept in the light; (9) normal seedling kept under window-glass in the dark, and (10) normal seedling, kept under window-glass in the light.

lengths of 320 to 390 $m\mu$ and kept in the dark, had attained an average length of 36 mm., 50 per cent. more than those kept under ultra-glass in daylight. Likewise seedlings covered by vitaglass which transmits wave-lengths down to 270 $m\mu$ had attained twice the growth in the dark that they had in the light. Although, here, the percentage of the lesser wave-lengths transmitted exceeded that of the ultra-glass, yet the inhibiting influence of the visible together with a possible coagulation by the lethal rays would appear to minimize the stimulative effect of the optimal region of lesser wave-length. The seeds within pot IIIb, irradiated for five minutes each day through window-glass, and kept in daylight, had a growth of 1 mm., while those in pot IVb, irradiated directly for the same period of time, and kept equally moist, had attained an average growth of 5 mm. Seedlings in pots IIIa and IVa, kept in the dark except for the five minutes of irradiation, attained a growth of 23 mm. and 19 mm. respectively, only slightly less than the seedlings grown under ultra-glass and vitaglass. Direct irradiation would appear to produce a lethal effect, for the growth in IVa did not quite equal that in IIIa. Then, too, growth of the seedlings in IIIa did not quite equal that of seedlings in IIa, in which the percentage of transmission of the optimal stimulative wave-lengths was greater.

In the normal control pots, Va and Vb, a conspicuous difference was apparent. Those seedlings kept constantly in the dark had attained an average length of 28 mm., while those kept constantly in daylight and under ordinary glass had attained at the end of eighty-eight hours a growth of

but 2 mm. In this case, the seedlings of the dark cabinet exceeded in length all others except those grown under the ultra-glass. If germination and growth are functions of the lesser wave-lengths, as they appear to be, we should expect the growth in pot IIIa, subjected to the ultra-violet irradiation, to exceed that in pot Va. Except for the brief periods of irradiation all conditions were identical. There is, however, a difference of 5 mm. in growth between seedlings grown under identical conditions except for this single variable factor. It is true that seedlings in IIIa received wave-lengths as low as 320 $m\mu$ during irradiation, but they were exposed to the visible light for a similar period. And it may be that such exposure was sufficient not only to inhibit the stimulative effects of the shorter rays, but to minimize as well the latent capacity for growth normally stored within a seed. The seeds in Va, given no stimulative exposures, and at the same time kept constantly from the visible rays, were slow in germinating; but the latent stored energy, undisturbed by the antagonistic effects of stimulators and inhibitors, could slowly but gradually come into expression.

Measurements taken at the end of each ensuing twenty-four-hour period for eight days showed that in each experimental condition those seedlings grown in the dark exceeded in length those similarly grown in the light. At the conclusion of the period of observation the greatest difference in the length of the seedling, when the light factor was the only variable, occurred in pots IIIa and IIIb. Here seedlings kept in daylight attained a growth only 5 per cent. of that reached by the seedlings kept in darkness; yet the exposure to the lamp was identical. In the case of those seedlings exposed to direct irradiation by the lamp and kept under ordinary glass, the growth of those kept in the light was only 15 per cent. of that attained by those kept in the dark. Of the controls, those kept in the light were 7 per cent. as long as those kept constantly in the dark. In the cases of the special filters, those seedlings grown under ultra-glass and kept in daylight were 57 per cent. of the length of those in the dark cabinet; while those under vitaglass and in daylight were 45 per cent. as long as those maintained in darkness.

Discussion

The attempt has been made to study the effect of wave-length on the germination and growth of the cucumber seed. Previously it has been shown that the lesser wave-lengths produce a stimulative effect on protoplasm. In studies on the hatching and growth of *Rana*³ we have shown that the violet and the ultra-violet spectral regions hasten the normal embryologic stages and that certain portions of greater metabolic activity respond more acutely

³ HIGGINS, GEORGE M., and SHEARD, CHARLES. Effects of ultra-violet radiation on the early larval development of *Rana pipiens*. Jour. Exper. Zool. 46: 333-343. 1926.

to the irradiation. The present experiments on germination have produced results that correspond with more or less exactness to those on the Amphibian ova. In studies on hatchability of the ova the spectral regions employed during the irradiation were not as closely delineated as in these observations on the germinating seeds. In these recent studies filters have been employed to restrict the percentage of wave-length transmission to known regions and thus to establish the influence of selective radiation.

On the basis of the observations and measurements recorded, all other conditions such as temperature and moisture being equal, it is evident that germination and growth are accentuated by radiation in the "near"-ultra-violet region (approximately 400 $m\mu$ to 300 $m\mu$). The seeds first to germinate were those exposed to rays transmitted by the ultra-glass filter. Those seeds within the normal control pot, which was kept constantly in the dark and thus subject to the same conditions, did not germinate until twenty-four hours later. Temperature was not a factor, for the ultra-glass employed did not transmit wave-lengths in excess of 400 $m\mu$, and thus we must conclude that the germination was induced by the lesser wave-lengths. In the case of the seeds grown under vitaglass, which transmits wave-lengths as short as 270 $m\mu$, seedlings were never as long at corresponding periods of time as those grown under the ultra-glass. Here, the percentage of transmission of the lesser wave-lengths is greater; but the somewhat lessened growth attained by the seedlings is due perhaps to two factors. Wave-lengths as low as 290 $m\mu$ and lower are known to be definitely lethal, and it is probably true that to a certain extent incipient coagulation induced by the exposure to the lethal rays had impeded germination. Likewise, visible light apparently serves to inhibit normal germination. So that in the case of the vitaglass the lessened growth may be due to the inhibiting effect of the visible light or the lethal effect of the lesser wave-lengths.

The radiation from the quartz mercury arc transmitted by window-glass (as short as 320 $m\mu$) seems to inhibit germination of seeds when they are subsequently exposed to solar radiation transmitted through window-glass. In darkness, however, the resident energy within the seed is augmented by such irradiation, so that germination occurs much more rapidly than it does in a similar seed unexposed to the rays of the lamp. Repeated exposures to radiation from the mercury lamp as short as 320 $m\mu$ serve to increase the rate of growth of the seedling, so that on the seventh day of experimental observation such seedlings exceeded in length those grown under the vitaglass. Here again, it would seem that the lethal effect of the lesser wave-lengths, which were filtered out by the ordinary glass and transmitted by the vitaglass, was the causal differential factor.

Direct exposures to the rays of the mercury-arc lamp are not ultimately beneficial. Germination, however, is accelerated, and as is to be expected,

a growth is attained, during the first forty hours, equal to that of seedlings under the vitaglass. Subsequent exposure to the direct rays, carrying with it the added lethal effect, is disastrous, for the rate at the end of the second period had decreased and the seedling had attained its maximal growth on the fourth day. Similar conditions obtain for seedlings directly irradiated and kept in the light. Germination occurred sooner than in the normal control group, and the seedling reached its maximal growth on the fourth day. The extent of growth of these seedlings, irradiated directly and kept in the light, is somewhat difficult to understand. A maximal growth of 8 mm. was attained on the fourth day, in contrast to a maximal growth of 3 mm. attained on the fifth day from those seeds irradiated through ordinary glass. The percentage of stimulating wave-lengths falling on the seeds was greater in the former case, and, at the same time, these seeds were irradiated with wave-lengths definitely lethal in character. One must conclude that the lethal effect was more than balanced by the stimulative effect of the optimal wave-lengths.

The normal control seedlings in the dark attained the greatest growth during the period of observation. Germination was somewhat retarded, however, and did not take place until the second period. Subsequent growth nevertheless was exceedingly rapid and during the third period the length exceeded that under the vitaglass, with its daily exposures to the stimulative rays. During the sixth period of observation, the length of the control seedling in the dark reached and surpassed that of those grown under the ultra-glass, thus exceeding in extent all other seedlings grown during the experiment. The control seeds in the light did not germinate until the fourth day and attained a maximal length of 8 mm. during the sixth day, exceeding by 5 mm. the maximal growth of seeds irradiated through ordinary glass and kept in the light. In other words, seedlings appear to thrive better when kept in daylight, if they are not exposed to brief periods of irradiation by the mercury lamp. When irradiated through window-glass, rays as short as 320 $m\mu$ are transmitted; but these may be rendered ineffectual by the long waves of the lamp which appear to be even more inhibitory than the visible radiations of daylight as transmitted by ordinary window-glass.

Conclusions

1. Selective irradiation of the cucumber seed modifies the time of its germination and rate of its subsequent growth.
2. Lesser wave-lengths in general appear to stimulate, while the greater wave-lengths inhibit germination.
3. Wave-lengths ranging from about 320 $m\mu$ to 390 $m\mu$ seem particularly effective in inducing growth.

4. Wave-lengths of 270 $m\mu$ to 320 $m\mu$ appear to be inhibitory in their action, delay the time and lessen the rate of growth, probably by reason of changes which, carried to their extreme, eventuate in coagulation of the seed albumin.

5. Some of the energy emitted by the lamp and absorbed by the seed may be rendered ineffective by subsequent exposure of the seed to the visible and near infra-red regions of interior daylight.

6. Certain wave-lengths of radiant energy are more potent in germination than temperature. With a constant temperature, germination and growth in the dark greatly exceed those in daylight as transmitted by ordinary window-glass.

7. A certain amount of energy, apparently produced under the action of lesser wave-lengths of sunlight, is normally stored up within seeds. Under proper conditions of light and moisture this energy induces germination.

8. Lesser wave-lengths of light act as stimulative agents which modify the control of endogenous processes and accelerate germination, while subsequent growth and development of the plant is doubtless a function of the visible or infra-red wave-lengths.

SECTION ON PHYSICS AND BIOPHYSICAL RESEARCH AND DIVISION OF EXPERIMENTAL
SURGERY AND PATHOLOGY,
MAYO CLINIC AND THE MAYO FOUNDATION,
ROCHESTER, MINNESOTA.

LENGTH OF LIFE OF SEED-PIECE ROOTS OF SUGAR CANE AND PROGRESS OF THE ROOTS IN THE SOIL AT DIFFERENT AGES OF GROWTH

H. ATHERTON LEE AND D. M. WELLER

(WITH FOUR FIGURES)

Introduction

In a previous paper¹ a method of determining quantitatively the distribution in the soil of the roots of sugar cane has been described, and the results of field studies showing such distribution have been presented. Further studies have been made since this previous publication which are recorded in the present paper.

As is perhaps commonly known, sugar cane is propagated on a plantation scale by vegetative cuttings called seed pieces. A seed piece of sugar cane, when it is first planted, puts out roots from the root bands at its nodes at the same time that its eyes or buds germinate. Each eye grows into an aerial shoot but it is some time before such an aerial shoot forms its own nodal roots. During this period such an aerial shoot obtains its nutrients from the vegetative seed piece and through the seed piece from the seed-piece roots. By referring to fig. 1, this can be more readily understood.

The bud of the cane cutting germinates to form an aerial shoot and at the same time the root eyes of the cutting germinate to form roots. The aerial shoot does not form its own roots until it has formed its first cane node and then produces roots from the root band at the node. Until these nodal roots of the stalk are formed, the cane plant functions on nutrients from the seed piece and from the seed-piece roots through the seed piece. Plant A, one month old, shows seed-piece roots formed almost exclusively, while Plant B, three months old, shows stalk roots preponderating over seed-piece roots.

The experiments recorded here show the period in the life of normal young cane plants during which these seed-piece roots function, and the period at which the new cane plant puts out its own nodal roots and functions independently of the seed piece and its roots. The downward progress of the roots into the different levels of soil is also shown from these studies.

¹ LEE, H. ATHERTON. The distribution of the roots of sugar cane in the soil in the Hawaiian Islands. *Plant Physiol.* 1: 363-378. 1926.

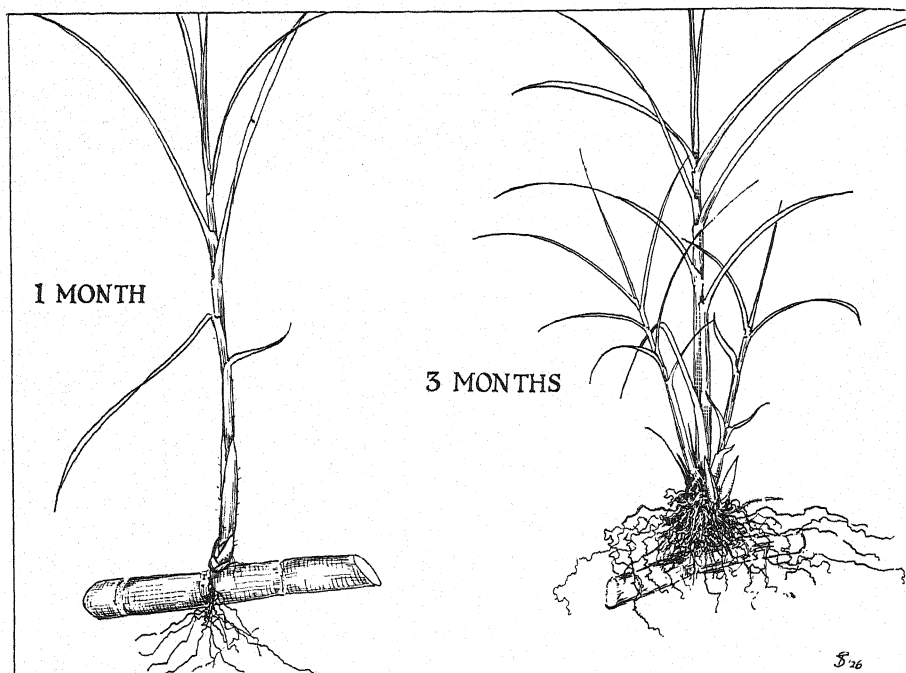


FIG. 1. The method of formation of roots in the early stages of the cane in the experiments.

Methods of study

Fifteen root-study boxes having the removable sides and horizontally placed wire netting, as previously described (*loc. cit.*), were planted each with one seed piece of the variety H 109. These seed pieces were selected for uniformity of length of internodes, diameter, and position on the stalk. Each seed piece consisted of three nodes with their accompanying three buds or eyes; of these three eyes, the two at the extremities were excised, leaving only one bud, or eye, to germinate for each seed piece. At time intervals of one month three of the boxes were taken in consecutive order, according to their position, the sides removed and the soil washed away from the roots of the cane in each box. The roots of the cane were thus left in correct position suspended on the wire netting.

At different levels in depth in the soil, beginning at the bottom and working upwards, the roots were cut off; thus all the roots below the 24-inch level were first cut off and collected. Next the roots between 16 and 24 inches in depth were cut at the 16-inch level, and collected; the roots between 8 and 16 inches in depth were next collected and finally the roots between the soil

TABLE I

WEIGHTS OF ROOTS FROM AERIAL SHOOTS AS COMPARED WITH WEIGHTS OF SEED-PIECE ROOTS
OF VARIETY H-109 AT DIFFERENT PERIODS OF GROWTH
AVERAGE WEIGHTS FROM THREE PLANTS OF EACH AGE

LEVELS IN DEPTH	CANE 1 MONTH OLD				
	AERIAL-SHOOT ROOTS		SEED-PIECE ROOTS		TOTAL BOTH CLASSES OF ROOTS
	gm.	Per cent.	gm.	Per cent.	gm.
Topmost 8 inches.....	0.036	4.0	1.094	96.0	1.130
8 to 16 inches.....	0.00	0.0	0.162	100.0	0.162
16 to 24 inches.....	0.00	0.0	0.025	100.0	0.025
24 inches downward	0.00	0.0	0.003	100.0	0.003
Totals	0.036		1.284		1.320
Percentages of class in total		2.7		97.3	

CANE 2 MONTHS OLD

	gm.	Per cent.	gm.	Per cent.	gm.
Topmost 8 inches.....	9.58	72.5	3.62	27.5	13.20
8 to 16 inches.....	2.66	88.3	0.35	11.7	3.01
16 to 24 inches.....	0.94	100.0	0.00	0.0	0.94
24 inches downward	0.33	100.0	0.00	0.0	0.33
Totals	13.51		3.97		17.48
Percentages of class in total		77.3		22.7	

CANE 3 MONTHS OLD

	gm.	Per cent.	gm.	Per cent.	gm.
Topmost 8 inches.....	96.75	98.2	1.77	1.8	98.52
8 to 16 inches.....	29.63	100.0	0.00	0.0	29.63
16 to 24 inches.....	14.11	100.0	0.00	0.0	14.11
24 inches downward	9.27	100.0	0.00	0.0	9.27
Totals	149.76		1.77		151.53
Percentages of class in total		98.8		1.2	

CANE 4 MONTHS OLD

	gm.	Per cent.	gm.	Per cent.	gm.
Topmost 8 inches.....	150.2	99.3	1.0	0.7	151.2
8 to 16 inches.....	48.6	100.0	0.0	0.0	48.6
16 to 24 inches.....	27.0	100.0	0.0	0.0	27.0
24 inches downward	24.0	100.0	0.0	0.0	24.0
Totals	249.8		1.0		250.8
Percentages of class in total		99.6		0.4	

TABLE I (*Concluded*)
CANE 5 MONTHS OLD

LEVELS IN DEPTH	AERIAL-SHOOT ROOTS		SEED-PIECE ROOTS		TOTAL BOTH CLASSES OF ROOTS
	gm.	Per cent.	gm.	Per cent.	gm.
Topmost 8 inches.....	229.0	99.7	0.6	0.3	229.6
8 to 16 inches.....	61.2	100.0	0.0	0.0	61.2
16 to 24 inches.....	25.4	100.0	0.0	0.0	25.4
24 inches downward	17.4	100.0	0.0	0.0	17.4
Totals	333.0		0.6		333.6
Percentages of class in total		99.8		0.2	

surface and the 8-inch level. In collecting these roots, those which emanated from the seed piece were carefully separated from the roots originating from the nodes of the aerial shoot or stalk. The separate root collections were then washed more carefully to remove all traces of soil, oven-dried and weighed.

The relation of seed-piece roots to nodal roots of the stalk

Table I shows the weights of the seed-piece roots as compared to the weights of the nodal roots of the cane stalks.

The results recorded in table I show that the cane plant functions entirely by the use of the roots from the seed piece for one month; at the end of one month 97.3 per cent. of the total roots originated from the seed pieces while only 2.7 per cent. of the roots originated from the stalks of the aerial shoots. At the end of two months the situation had changed considerably, only 22.7 per cent. of the roots having arisen from the seed piece as compared with 77.3 per cent. of the roots from the stalks of the aerial shoots. At the end of the third month the situation was completely reversed, with only 1.2 per cent. of the roots emanating from the seed piece and 98.8 from the stalks of the aerial shoots. Thereafter the roots from the seed piece constituted but a negligible proportion of the total roots. The relation of the weights of seed-piece roots to the weights of aerial-shoot roots at different ages of growth is shown graphically in fig. 2.

It is of interest that this change in the proportion of nodal stalk roots to seed-piece roots was not due alone to the increased weight of the nodal stalk roots; after the second month the seed-piece roots did not increase but actually decreased in weight. At the end of the fifth month the seed-piece roots weighed but 0.6 gm. as compared to 333 gm. of nodal stalk roots, amounting to but 0.2 per cent. of the total roots. Since the seed-piece roots

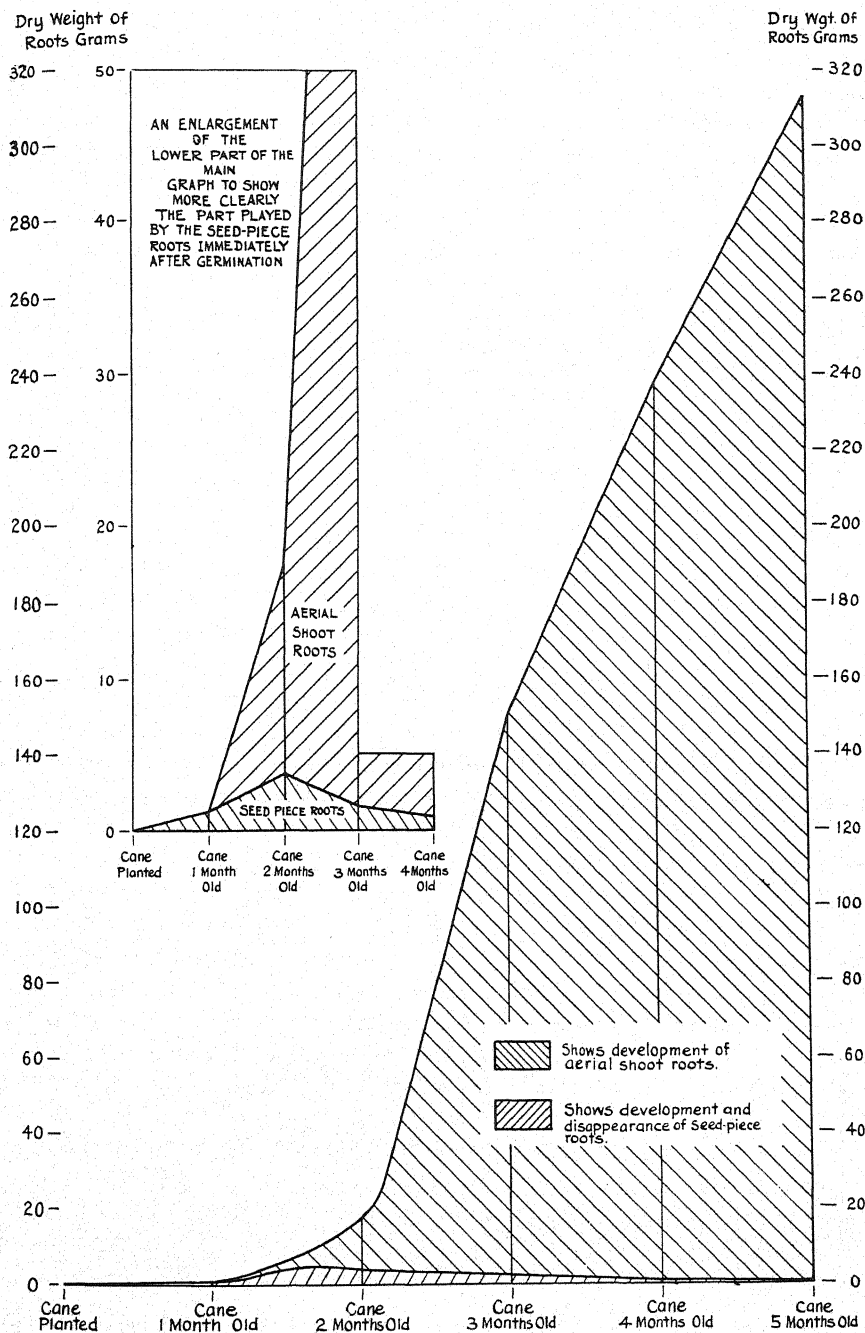


FIG. 2. The dry weight increase of seed-piece roots as compared to aerial-shoot or stalk roots at different ages in the cane growth.

are not true seminal roots, there is no correlation, nor would any be necessarily expected, between the persistence of the seminal roots of cereal crop plants such as wheat as developed by KRASSOVSKY,² and the length of life of roots from these vegetative cuttings of sugar cane.

Therefore under normal conditions *apparently the seed-piece roots alone furnish the nutrients for the aerial shoot for the first month. After the first month and to the end of the second month there is a transition period during which the burden of supplying nutrients shifts from the seed-piece roots to the nodal stalk roots. At the end of the third month and thereafter the burden of supplying nutrients rests almost entirely on the nodal stalk roots since the seed-piece roots have disappeared.*

Discussion

It has been argued from these data that fertilizers should not be applied to the cane until the stalk puts out its own roots, and that fertilizers applied to seed-piece roots will only stimulate roots which will very shortly die and roots will be built up which will be wasted. More careful analysis indicates that there are reasons for early applications of fertilizers which outweigh the foregoing considerations. The aerial shoot cannot form its own roots until it has formed at least one node and the accompanying root band at that node. Thus fertilizers applied early will stimulate the formation of the first node on the aerial shoot and hasten the formation of the first nodal roots. That part of the fertilizer which is not used by the seed-piece roots will remain for utilization by the aerial-shoot roots. That part of the fertilizer used in the formation of the seed-piece roots will not be lost but on the decay of the seed-piece roots will be returned to the soil. One would expect therefore that experiments with nitrogen and potash as well as phosphoric acid, in the furrow, would possibly yield interesting results.

In connection with root-rot studies there is an important conclusion to be drawn, *that one should discriminate between rots of the seed-piece roots after the first month of growth, and rots of the roots from the cane stalk; the decomposition of the former would seem to be a more or less natural feature of the life processes of the cane plant while, of course, rots in the stele of the roots of the cane stalk would be decidedly abnormal.*

The progress in growth of the roots at different ages

In addition to the data showing the comparative weights and proportions of seed-piece roots and stalk roots, data were obtained showing the development of roots of both classes in the different levels in depth in the soil at different ages of the cane. These data are recorded in table II.

² KRASSOVSKY, IRENE. Physiological activity of the seminal and nodal roots of crop plants. Soil Science 21: 307-322. 1926.

TABLE II
WEIGHT OF CLEAN OVEN-DRY CANE ROOTS AT THE DIFFERENT LEVELS IN DEPTH OF THE SOIL AT DIFFERENT PERIODS IN THE
AGE OF THE CANE

LEVELS IN DEPTH	AGE OF THE CANE									
	1 MONTH		2 MONTHS		3 MONTHS		4 MONTHS		5 MONTHS	
	WEIGHT	PROPOR- TION	WEIGHT	PROPOR- TION	WEIGHT	PROPOR- TION	WEIGHT	PROPOR- TION	WEIGHT	PROPOR- TION
Topmost 8 inches.....	gm. 1.130	Per cent. 85.6	gm. 13.20	Per cent. 75.5	gm. 98.52	Per cent. 65.0	gm. 151.2	Per cent. 60.3	gm. 229.6	Per cent. 68.8
8 to 16 inches.....	0.162	12.2	3.01	17.2	29.63	19.5	48.6	19.4	61.2	18.3
16 to 24 inches.....	0.025	1.9	0.94	5.3	14.11	9.3	27.0	10.7	25.4	7.6
24 inches downward	0.003	0.3	0.33	1.9	9.27	6.1	24.0	9.5	17.4	5.2
Totals	1.320	100.0	17.48	99.9	151.53	99.9	250.8	99.9	333.6	99.9

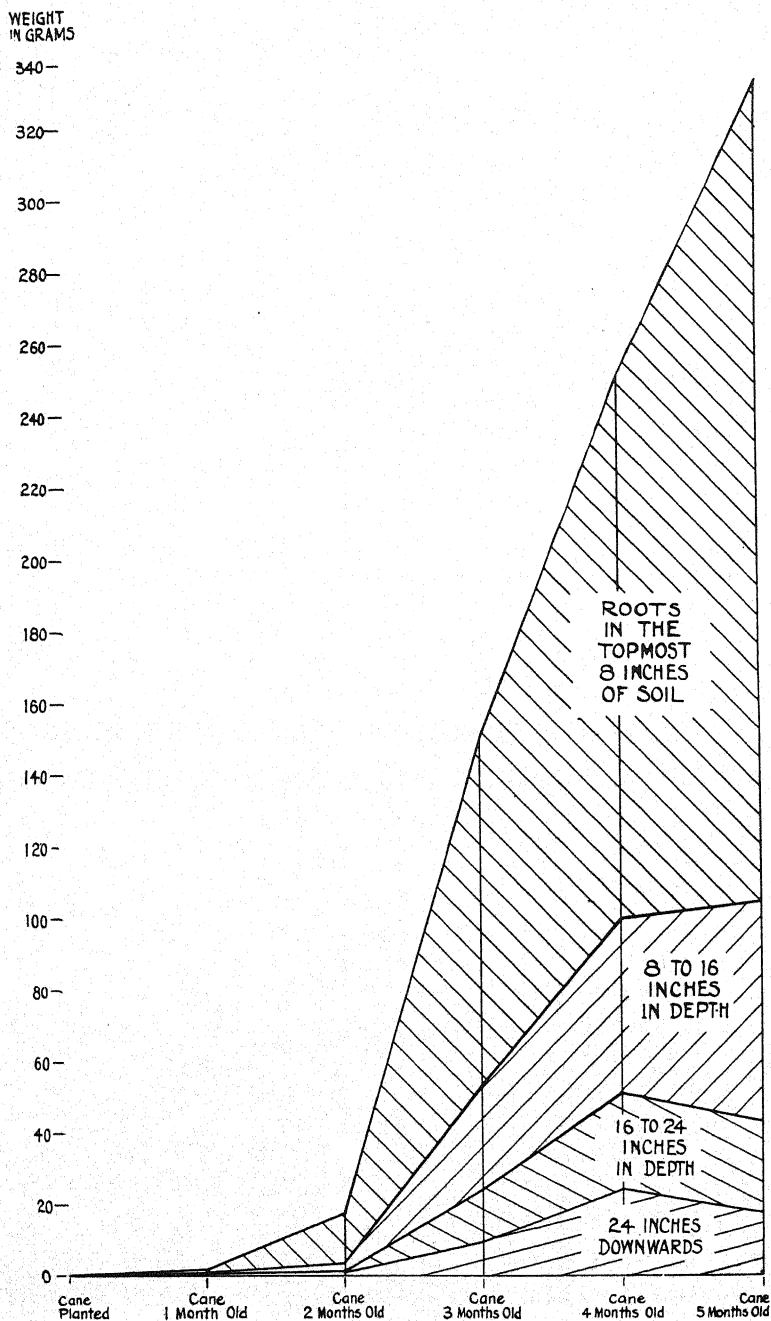


FIG. 3. Dry weight of roots at the different levels in depth in the soil at different periods of growth.

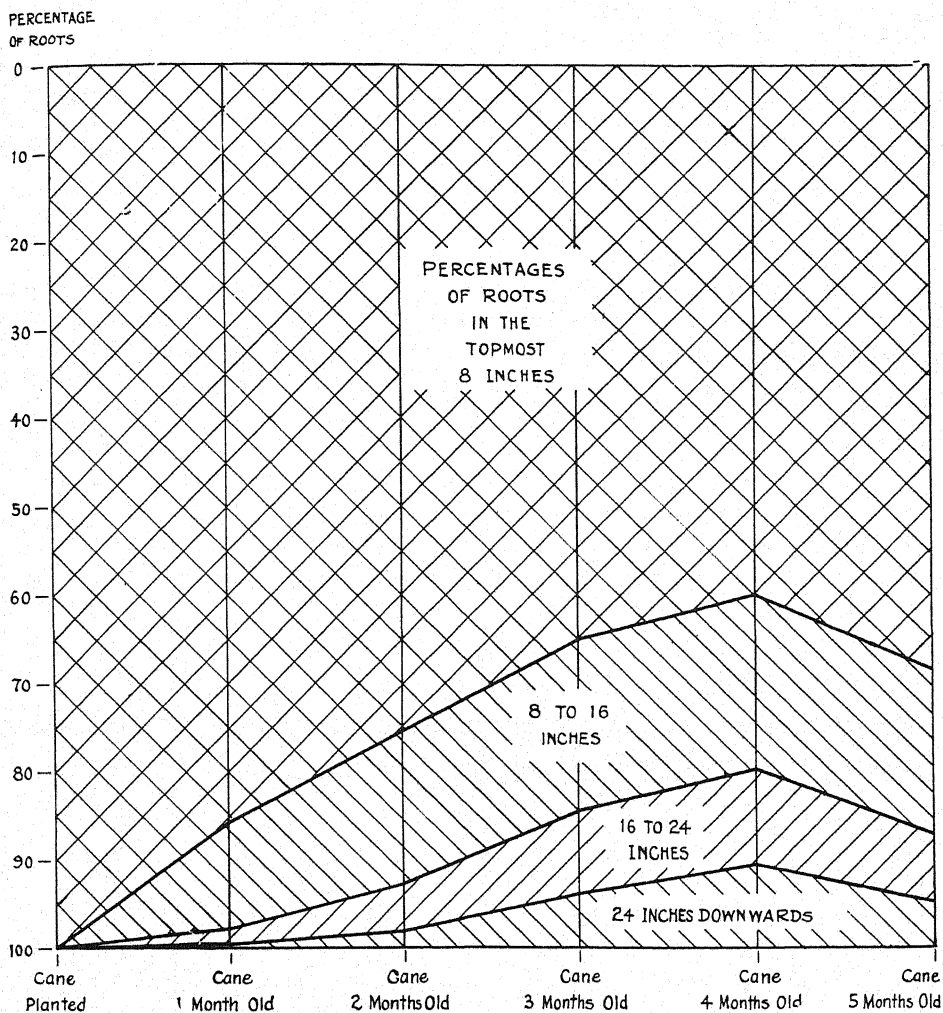


FIG. 4. The percentages of roots of plants of H 109 at different levels in depth in the soils at different ages up to 5 months from planting.

The figures in table II show the combined weights of both seed-piece roots and the roots formed at the nodes of the aerial shoot; the weights, given in grams, are the averages of three plants of each age.

Examining first the figures showing weights, the results indicate what would naturally be expected, that the root weights increased with age, and that the upper levels of the soil were first penetrated and then successively

the lower levels. The weights of the roots in the different levels in depth at the different ages in growth are shown graphically in fig. 3.

The figures concerning percentages of roots are fully as important as the figures for root weights, for in the application of fertilizers and irrigation water, one wishes to place such applications where the largest proportion of the roots exist, and total weights are not as relevant in such questions as are the percentages of roots. If one now refers to the percentages of roots in the different levels in depth in the soil as shown in table II, it can be seen that starting with 85 per cent. of the roots at the end of the first month *the proportions of the roots in the topmost 8 inches of soil gradually decreased until about 60 per cent. of the roots were found in this level; the curve of the decrease then leveled off and it is expected that the plants maintain somewhere between 55 and 70 per cent. of their roots in this stratum until maturity*, at least the results of field root studies (*loc. cit.*) support such a view. At the same time *the percentages of the roots in the lower strata increased to a given proportion and the curve of increase then appeared to level off giving a more or less fixed proportion of the roots through to maturity*. The graph shown in fig. 4 illustrates this approach to fixed proportions of root quantities in the different levels in the soil, after the first few months of growth.

Discussion

It seems to us established from these studies, supported by the field studies previously reported, that water and nutrients, to reach the greatest proportion of roots, should be directed towards the uppermost 16 inches of soil where more than 75 per cent. of the roots usually exist. That tillage and cultivation also need only be shallow seems to us not entirely warranted. As a result of observations during this work on roots we have come to the opinion that, given optimum moisture and nutrients, the outstanding factor for formation of the important secondary roots with their large proportion of feeding surfaces is soil aeration. We do not have quantitative data to support this opinion; our views are based upon field observations only and we present these views as opinion only. If this opinion is correct then deep tillage and organic matter would improve aeration and such improved aeration would increase the feeding surfaces of the roots, thus indirectly increasing cane tonnage. This suggests the desirability of field experiments to test root formation and cane tonnage with increased soil aeration as compared with control conditions.

Summary

1. Based on root weights, a normal sugar cane plant obtains its nutrients during the first month of growth from the seed piece and through

the seed piece from the seed-piece roots. At the end of one month seed-piece roots averaged 97.3 per cent. of the total roots of the plant, and roots from the new aerial shoot or stalk only 2.7 per cent. of the total.

2. At the end of the second month the seed-piece roots constituted only 22.7 per cent. of the total roots, while roots from the new aerial shoots constituted 77.3 per cent. of the total. At the end of the third month but 1.2 per cent. of the total roots were seed-piece roots and 98.8 per cent. of the roots were given off from the aerial shoot or stalk. After this period the seed-piece roots continued to reduce in weight and constituted a negligible proportion of the total roots.

3. A comparison of root weights at different levels in depth in the soil at different periods of growth showed that the quantity of roots in the upper levels of soil increased with the age of the plants; but, while the uppermost roots in the first month of growth constituted fully 85 per cent. of the total roots, yet this percentage gradually decreased during the following month of growth, until only 50 to 75 per cent. of the roots existed in the topmost 8 inches of soil. From that time on, the proportion of roots in the topmost levels of soil became more or less constant.

4. The results presented here suggest further experiments and also give us new points of view in interpreting the results of experimental work dealing with various agricultural practices. There are a number of obvious applications of these results to tillage, cultivation and fertilizer practices.

EXPERIMENT STATION,

HAWAIIAN SUGAR PLANTERS' ASSOCIATION,

HONOLULU, HAWAII.

FACTORS AFFECTING THE COMPOSITION OF DATES

M. T. FATTAH AND W. V. CRUESS

It is of interest to know whether the composition of the more important commercially grown varieties of dates is affected by the variety and locality where grown and what important changes occur during the ripening process. In some localities, as in Mesopotamia and North Africa, dates mature completely on the trees; in others, because of lack of sufficient heat units during the ripening season, artificial ripening or "processing" is required. Some varieties if allowed to ripen completely on the tree "mummify" and are best if picked slightly unripe and artificially ripened after removal from the tree.

Moisture, total sugars and tannin were taken in our studies as the principal indices of maturity and quality of the dates used.

Effect of locality

Dates are grown in many tropical and sub-tropical countries, among them California and Mesopotamia, the two districts compared in our studies.¹ Growing conditions in these two regions differ considerably. Supposedly mature dates from the two regions were analyzed for moisture, sucrose, reducing sugars and tannin. Some of the data are given in table I.

The Mesopotamian dates were consistently higher in total sugars than were the same varieties grown in California. This possibly means that the dates grown in Mesopotamia ripened fully on the trees, whereas in California, where the heat units during the growing season are said to be somewhat less, the fruit did not reach full maturity or was harvested somewhat sooner than in Mesopotamia in order to reach the market early.

Sugar was determined by the SHAFFER-HARTMANN² volumetric method. Tannin³ was determined by titration with dilute standard KMnO_4 , using indigo carmine indicator.

Effect of maturity

Our studies on this phase of the problem were limited to a rather narrow range of maturity, from what might be termed "commercially green" to

¹ Samples of dates were kindly supplied by the Tropical Date Garden and the U. S. Dept. of Agr. Date Garden, of California, and by Fattah and Sons, of Bagdad, Mesopotamia.

² SHAFFER, P. A., and HARTMANN, A. F. The iodometric determination of copper and its use in sugar analysis. *Jour. Biol. Chem.* 45: 349-390. 1921.

³ See Official and tentative methods. *Assoc. Official Agr. Chemists*, 2nd ed., p. 367. 1925.

TABLE I

MOISTURE, SUCROSE, REDUCING SUGARS AND TANNIN IN FLESH OF DATES FROM MESOPOTAMIA AND CALIFORNIA

PERCENTAGE EXPRESSED ON DRY WEIGHT BASIS

SAMPLE	MOISTURE CONTENT	REDUCING SUGARS	SUCROSE	TOTAL SUGARS AS INVERT SUGAR	TANNIN
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Kadrawi, Besia, Mesopotamia	13.9	74.20	0.00	74.20	0.00
Kadrawi, U. S. Date Garden, Calif.	15.4	72.12	1.25	73.43	0.02
Kadrawi, Tropical Date Garden, Calif.	31.6	70.63	2.36	73.11	0.05
Khustawi, Bagdad, Mesopotamia	15.5	77.20	1.52	78.80	0.00
Khustawi, U. S. Date Garden, Calif.	14.6	72.13	0.53	72.66	0.00
Mactum, Bagdad	15.1	80.2	0.00	80.20	0.00
Mactum, U. S. Date Garden, Calif.	17.5	72.15	0.34	72.50	0.00
Zahidi, Bagdad	17.7	83.40	0.76	84.20	0.00
Zahidi, Tropical Date Garden, Calif.	15.6	72.32	1.50	73.89	0.00

"commercially ripe." In all cases the fruit had reached full size. Included also are a few analyses giving a comparison of mummified and soft ripe dates. A few analyses selected from those made are given in table II.

The green dates of all varieties contained considerable quantities of sucrose; with ripening, most of this sugar disappeared (probably by inversion) except in the Deglet Noor variety. The tannin in the green dates was much higher than in the ripened dates.

The mummified sample of Zahidi dates from Mesopotamia is particularly interesting. Although tree ripened, its composition is similar to that of an unripe date. It has 13.11 per cent. of sucrose and about 0.23 per cent. tannin contrasted with 0.76 per cent. sucrose and complete absence of tannin in the soft ripe dates of this variety. Observation shows that dates that mummify on the tree ripen first at the blossom end and ripening proceeds gradually toward the calyx end of the date. In the extremely hot dry air of Bagdad, apparently the calyx end of this variety becomes so dry that ripening is arrested and it remains somewhat immature. Qualitative tests for soluble tannin on the cut surface of longitudinal sections of the mummified Zahidi dates showed considerable tannin in the flesh of the calyx end and none to very little in the blossom end.

TABLE II

COMPARISON OF COMPOSITION OF FLESH OF GREEN, MUMMIFIED AND SOFT RIPE DATES
PERCENTAGE ON DRY WEIGHT BASIS

SAMPLE	MOISTURE	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	TANNIN
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Berhi, Tropical Date Garden, Calif., green	53.8	35.89	16.40	53.15	1.64
Berhi, Tropical Date Garden, Calif., medium ripe	27.8	46.93	15.80	63.56	0.10
Berhi, Tropical Date Garden, Calif., soft ripe	19.7	72.53	0.38	72.93	0.00
Deglet Noor, U. S. Date Garden, Calif., green	42.2	12.60	20.50	41.10	1.90
Deglet Noor, U. S. Date Garden, Calif., medium ripe	29.3	24.70	28.22	54.40	0.21
Deglet Noor, U. S. Date Garden, Calif., soft ripe	18.4	38.20	42.27	82.70	0.20
Deglet Noor, U. S. Date Garden, Calif., soft ripe	20.3	42.00	28.46	71.90	0.20
Hallawi, Tropical Date Garden, Calif., green	52.5	22.20	6.26	28.78	0.50
Hallawi, Tropical Date Garden, Calif., medium ripe	27.0	58.57	1.12	59.74	0.00
Hallawi, Tropical Date Garden, Calif., ripe	21.6	72.62	0.25	72.88	0.00
Kadrawi, Tropical Date Garden, slightly green	48.2	39.80	26.59	67.70	0.60
Kadrawi, Tropical Date Garden, medium ripe	32.20	43.20	24.40	68.51	0.20
Kadrawi, Tropical Date Garden, ripe	31.6	70.63	2.36	73.11	0.05
Zahidi, Tropical Date Garden, Calif., green	53.8	16.72	13.34	30.80	0.70
Zahidi, Tropical Date Garden, Calif., medium ripe	25.9	39.42	12.97	53.07	0.02
Zahidi, Tropical Date Garden, Calif., ripe and partly mummified	17.7	74.25	1.63	75.95	0.00
Zahidi, Bagdad, soft ripe	17.70	83.40	0.76	84.20	0.00
Zahidi, Bagdad, well mummified	14.40	66.80	13.11	76.80	0.23

The above explanation of the presence of appreciable amounts of sucrose in mummified dates does not apply, however, to several samples of Deglet Noor variety that were examined. Although the mummified Deglet Noor dates were rather hard in texture and contained considerably less total sugars than the soft ripe dates, they contained no more tannin than the soft ripe dates. FREEMAN⁴ also reported lower total sugar content in mummified than in soft ripe Deglet Noor dates. VINSON has studied the changes occurring in this and in other varieties throughout the growing and ripening period, the comparison being made between a "cane sugar" variety, the Deglet Noor, and various "invert sugar" varieties, principally seedlings.

Effect of variety

The composition of several of the leading varieties of dates is given in tables I and II. They may be grouped in two classes: (1) those high in cane sugar, and (2) those high in invert sugar and low in cane sugar. The Deglet Noor is the principal variety in class 1; most other commercially grown varieties fall into class 2, although when green, VINSON⁵ believes all varieties contain appreciable quantities of cane sugar. Our own observations indicate that this is true of the varieties examined. A few additional analyses

TABLE III
ADDITIONAL ANALYSES SHOWING EFFECT OF VARIETY ON COMPOSITION OF DATES
PERCENTAGE OF DRY WEIGHT BASIS

SAMPLE	MOISTURE	INVERT SUGAR	SUCROSE	TOTAL SUGAR	TANNIN
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Ashrasi, Mesopotamia	13.9	66.90	5.98	73.20	0.59
Azrak, Mseopotamia	15.7	69.30	0.28	69.60	0.00
Banawsha, Mesopotamia	15.1	65.60	2.09	67.69	0.00
Degal, Mesopotamia.....	14.1	59.20	1.62	60.90	0.00
Duck El Badam (mummified), Mesopotamia	10.4	39.00	26.40	66.80	1.80
Kadrawi, Mesopotamia	13.9	74.20	0.00	74.20	0.00
Khalal Matbookh,* Mesopotamia	8.8	22.5	33.63	57.9	0.76

* Cooked immature dates.

⁴ FREEMAN, G. F. Ripening dates by incubation. Arizona Exp. Sta. Bull. 66: Part II. 1911.

⁵ VINSON, A. E. Arizona Exp. Sta. Bull. 66: Part I. 1911.

showing the effect of variety are given in table III. Of particular interest is the last sample in this table, that of dates that were cooked and dried when slightly immature. Note their low total sugar, high sucrose, and high tannin content.

Changes in composition during incubation at 130° F.

As might be expected immature dates held at 130° F. ripened rapidly with loss of tannin and inversion of sucrose, as shown in table IV.

TABLE IV

CHANGES IN SUGARS AND TANNIN DURING INCUBATION OF IMMATURE BERHI DATES AT 130° F.

TIME	REDUCING SUGARS	SUCROSE	TANNIN
Hours	Per cent.	Per cent.	Per cent.
0	35.89	16.40	1.64
48	43.46	8.75	.03
96	48.64	3.52	.00

After 96 hours the dates were well softened, translucent, amber in appearance and of good eating quality. In this test (repeated with several other varieties with similar results) no attempt was made to remove excess moisture from the dates and they decreased but little in moisture content during the incubation.

TABLE V

CHANGES IN SUGARS, TANNIN AND MOISTURE DURING DEHYDRATION OF BERHI DATES AT 120° F.

PERCENTAGES ON DRY WEIGHT BASIS

TIME	MOISTURE	INVERT SUGAR	CANE SUGAR	TOTAL SUGAR	TANNIN
Hours	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
0	53.8	35.89	16.40	52.29	1.64
4	43.3	39.80	12.32	52.12	1.10
8	42.5	39.84	12.21	52.05	0.50
12	41.9	40.44	11.51	51.95	0.20
16	39.6	42.22	9.62	51.84	0.11
20	36.9	44.30	7.48	51.78	0.05
24	31.5	47.17	4.53	51.70	0.00
28	29.8	48.96	2.67	51.63	0.00
32	28.5	49.62	0.92	50.54	0.00

Changes during dehydration at 120° F.

Dates are often partially dehydrated before packing. In order to follow the changes in composition occurring during this treatment, Berhi dates (slightly immature) were dried in a blast of air (velocity 500 feet per minute) on screen trays at 120° F. Samples were analyzed at intervals, as given in table V.

Just as during incubation, sucrose decreased greatly and soluble tannin disappeared. After 32 hours the dates were in excellent eating condition. Dehydration was apparently superior to incubation in point of time required, and gave a better product in this test.

Ripening in various gases and vapors

Dates were ripened in various gases and vapors, including CO₂, O₂, CS₂, CHCl₃ and C₂H₅OH. Those ripened in CO₂ were normal in all respects and satisfactory in every respect. The changes occurring in sugars and tannin were typical of those in other gases or vapors and are given in table VI.

TABLE VI
CHANGES OCCURRING DURING RIPENING OF BERHI DATES IN CO₂
PERCENTAGE ON DRY WEIGHT BASIS

TIME	REDUCING SUGAR	SUCROSE	TANNIN
Hours	Per cent.	Per cent.	Per cent.
0	35.89	16.40	1.64
48	40.62	11.62	0.50
96	44.76	7.45	0.00

Dates ripened in O₂ were dark brown in color and of poor flavor. Incubation or dehydration at 120–130° accomplished the desired results more satisfactorily than ripening in gases. Dehydration is superior principally in that it permits control of the moisture content of the ripened fruit.

Summary

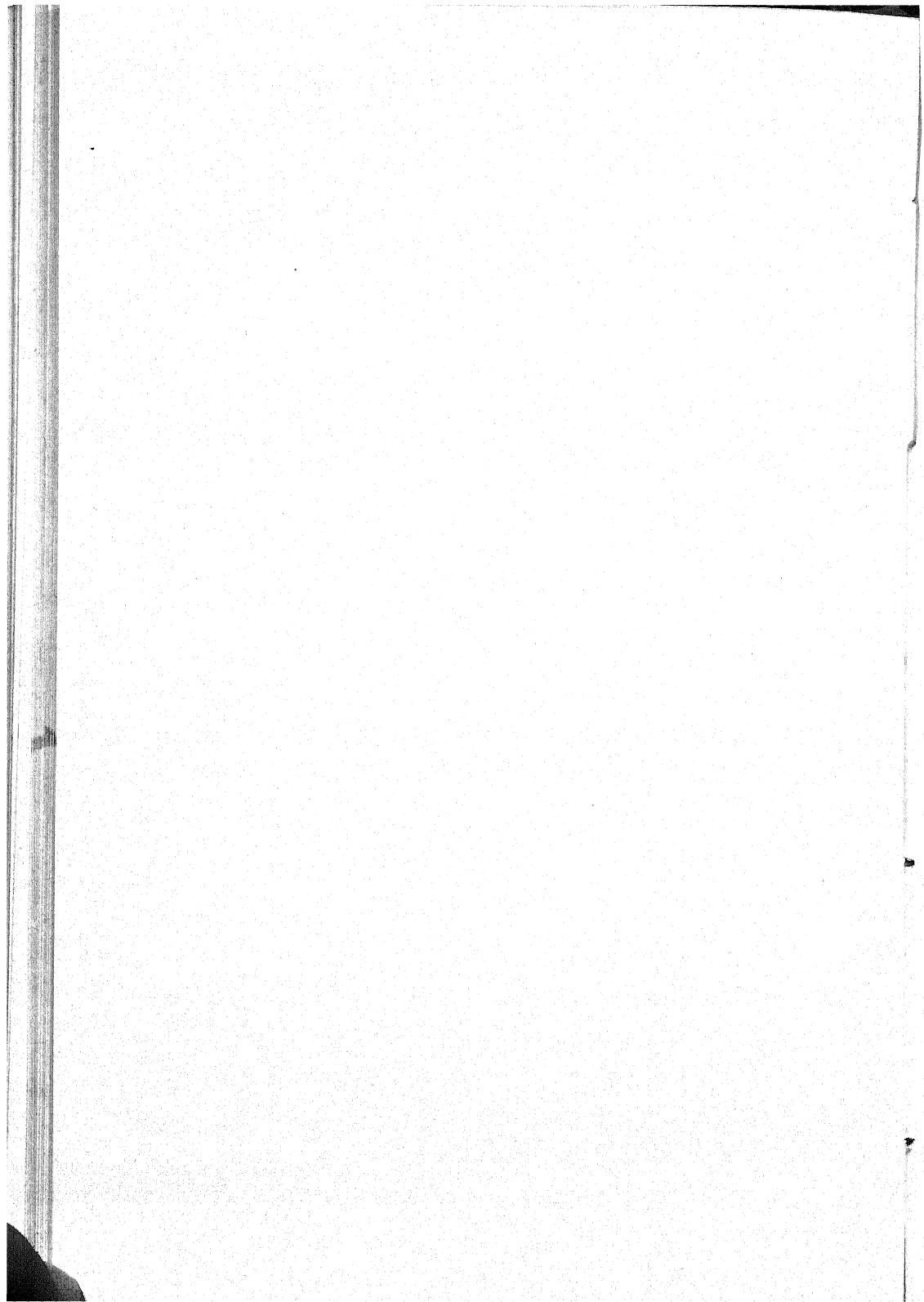
1. Dates from Mesopotamia used for comparison in this investigation were on the average higher in total sugars on the dry basis and lower in moisture content than the same varieties grown in California, owing perhaps to more favorable ripening conditions (temperature) in Mesopotamia.
2. There was found to be considerable difference in the total sugar content (on dry basis) of different varieties; in some cases this was apparently caused by arrested ripening by mummification (drying on the tree). The Deglet Noor was consistently high in sucrose; most other varieties were low

in this constituent when ripe. All unripe samples of all varieties examined contained considerable sucrose; this decreased greatly during ripening except in the Deglet Noor variety.

3. Soluble tannin was found to decrease markedly during ripening under various experimental conditions, such as during incubation, dehydration, and during storage in various gases and vapors.

4. Dehydration at 120° F. was the most satisfactory means of artificial ripening used in our experiments.

FRUIT PRODUCTS LABORATORY,
UNIVERSITY OF CALIFORNIA



BRIEF PAPERS

THE EFFECT OF ETHYLENE ON THE RESPIRATION OF BANANAS DURING RIPENING

(WITH ONE FIGURE)

In a paper from this laboratory presented at the Kansas City meeting of the American Association for the Advancement of Science, 1925, it was shown that ethylene doubled or trebled the production of carbon dioxide by celery for a short time after application, and that subsequently the rate fell off to a value below the normal respiratory rate at the same temperature.

Continuing these studies on the physiological influence of unsaturated hydrocarbons in ripening green fruits and vegetables, we have followed the rate of carbon dioxide production by bananas during ripening.

The fruit was placed in sealed glass containers provided with inlet and exit tubes. Suitable wash bottles were inserted to free the incoming air from CO_2 and to keep the air saturated with water vapor. The rate of carbon dioxide production was measured by means of the conductivity cell which was described in the issue of *PLANT PHYSIOLOGY* for April, 1926. The whole train of apparatus was kept in a constant temperature bath at 25°C . The ADAMS arrangement for the conductivity apparatus was used.

One or two bananas were usually used and the tests lasted from about five to fifteen hours. They were given one or more doses of ethylene carefully measured with a micro-gas burette. The dose was one part of ethylene to one thousand parts of air, since this was the concentration which had been found best suited to ripen bananas. The ethylene was allowed to act for fifteen to twenty minutes and then the aspiration was resumed. The air was aspirated from the container for fifteen to thirty minutes before passing it through the cell, to remove ethylene and the CO_2 which had been liberated during the period of treatment. Conductivity readings were taken every fifteen to thirty minutes thereafter for one or two hours, and if a second dose of ethylene was given to the same specimen, the procedure was repeated. The asterisks on the graph (fig. 1) indicate the points of treatment with ethylene. In all cases the rate of respiration expressed in milligrams of CO_2 per hour was doubled or trebled within a few minutes and then the rate fell off to a value lower than normal.

Bananas from the same bunch and run simultaneously with the treated bananas, although showing some fluctuation in rate, never showed the same

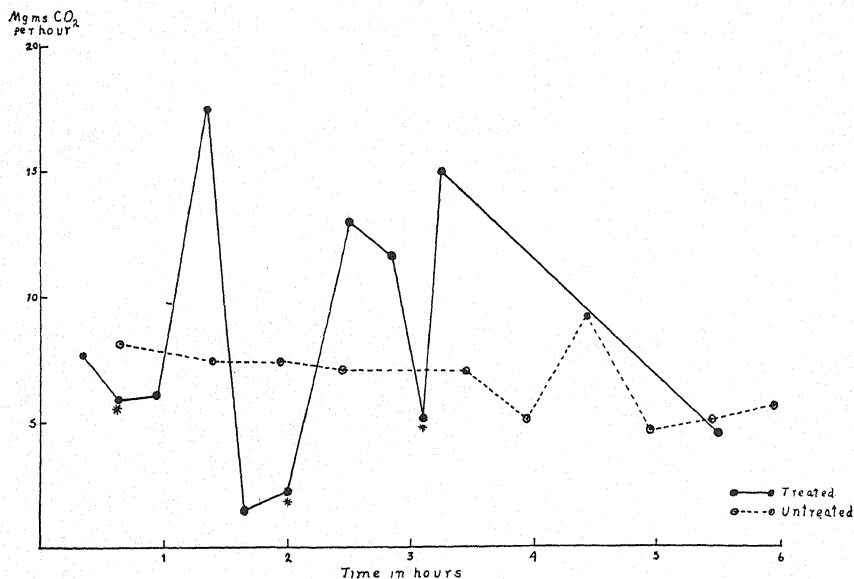


FIG. 1. Respiratory rate in bananas. Ethylene 1-1000 administered at the points indicated by asterisks.

high rates or minima after the passing of the peaks of the curve that the treated bananas did.

Since the trend in all cases tried was the same, it was deemed best to show the graph of one typical treated banana and of one check run simultaneously under exactly the same conditions to illustrate the point rather than attempt to graph several runs on the same paper. This one is typical of many other curves, using Le Gros Michael variety and Cavendish bananas.

The method which was used allowed for the rapid determination of the rate of CO_2 production over a few minutes; consequently it was possible to follow the rapid rise and fall of the respiratory rate better than could be done by the method employed by DENNY¹ on lemons. The high initial rate a few minutes after administration of the ethylene followed by a rapid fall to below normal may be due either to the increase of oxidation or to increase in the permeability of membranes allowing the diffusion of the CO_2 already present in the cells. The rise after the second dose of ethylene seems to indicate an increase in oxidation rate rather than permeability change. Evidently this stimulation wears off within less than an hour.

¹ DENNY, F. E. Effect of ethylene upon respiration of lemons. Bot. Gaz. 77: 322-329. 1924.

The rather rapid removal of ethylene by oxidation, as in the animal body after anaesthesia, offers an explanation for this. Continuous application of the ethylene seems necessary to continued increase in respiration.

Analyses made on treated and untreated bananas show that the treated bananas have one fifth to one fourth more sugar in them than the untreated bananas and that the starch content is proportionately decreased. The activity of the diastatic enzymes as well as the respiratory enzymes is increased by ethylene. Whether this is due to the cell permeability being increased, thereby making it easier for the enzymes and substrate to come together, and to facilitation of the intake of oxygen, or whether ethylene and propylene act as enzyme activators or actually increase the amount of the enzymes, we are now attempting to determine.—L. O. REGEIMBAL, G. A. VACHA, AND R. B. HARVEY, *The University of Minnesota*.

AN EFFECTIVE LABORATORY DRIER

(WITH ONE FIGURE)

A rather extended use of the phenol-disulphonic acid method for the determination of soil nitrates led the authors to experiment with various methods of speeding up the necessary step of evaporating aqueous extracts to dryness. The drier finally evolved (fig. 1) has proven fully satisfactory

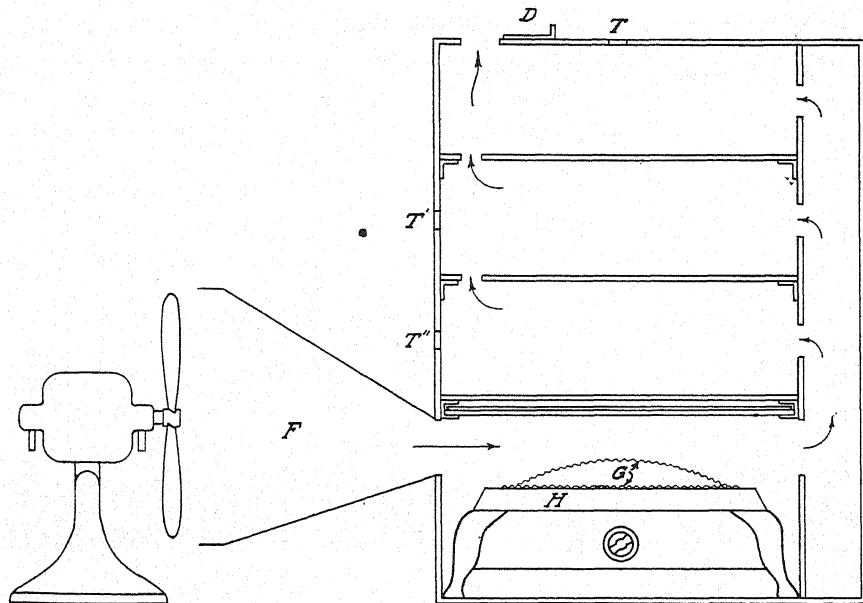


FIG. 1. Vertical section of laboratory drier. Description in text.

not only for that purpose, but also for the rapid drying of green plant tissues for analysis. The principle of operation will be obvious from the illustration. Its important advantages are (1) rapidity, (2) all parts of the drying chamber having very closely the same effectiveness. Details of size and construction may be varied to suit individual needs and preferences, but a brief description will be given of the model we have now in use.

A galvanized iron box, made by a local tinsmith, is lined with asbestos sheeting. The shelving and perforated partition shown at the right are made of asbestos slate (transite) held in place by light angle-irons and stove-bolts. A rapid air draft is provided by a 9-inch desk fan blowing through the funnel *F*, the path of the air being indicated by the arrows. The air passes across *H*, an electric hot-plate, into a narrow chamber at the right, from which it enters the drying chamber through 5 circular openings, 1.5 inches in diameter, opposite the space above each shelf. The second and third shelves do not run the full width of the drying chamber, but leave an air-gap at the left, bridged by light metal pieces (not shown in the figure). A set of air-holes at the top left are fitted with a damper *D* for controlling the draft. The shelf-space is 14 inches square, with 4.5 inches between shelves. With the draft full open and the hot-plate turned to "high" (1,000 watts), the temperature of the drying chamber runs 61° to 63° C. Thermometers are inserted at *T*, *T'* and *T''*, to check the various shelves. It was found necessary to protect the bottom shelf with two extra layers of asbestos sheeting, also to cut down direct radiation by two layers of wire gauze *G* over the hot-plate, to secure the same temperature on all shelves. The narrow chamber at the right is permanently closed in front by the wall of the box. The drying chamber is closed by a glass door, and the hot-plate by a piece of transite containing an opening for the switch. Over both the latter is fitted an outside door of galvanized iron.

Thermostatic control can be added at any time. It has not so far been necessary, as the drier has shown no tendency to vary more than a couple of degrees. In some laboratories it would no doubt be advisable to pass the entering current of air over a moisture absorbing agent. The low humidity of our Alberta atmosphere makes this unnecessary for ordinary purposes.—R. NEWTON AND W. H. COOK, *University of Alberta*.

NOTES

Officers for 1927-1928.—The Secretary of the American Society of Plant Physiologists has announced the results of the recent annual election as follows: President, Professor CHARLES A. SHULL, the University of Chicago; Vice-President, Dr. W. E. TOTTINGHAM, the University of Wisconsin, Madison, Wisconsin. The Secretary-Treasurer, Dr. S. V. EATON, the University of Chicago, was elected last year, and remains in that office.

Midwest Regional Meeting of the American Chemical Society.—The Seventh Midwest Regional Meeting of the A. C. S. was held at Chicago, May 27-28, 1927. The sessions were held in the Kent Chemical Laboratory of the University of Chicago. The meeting was a very valuable one, and was rendered notable by the award of the Willard Gibbs Medal to Dr. JOHN J. ABEL, distinguished biochemist and pharmacologist of Johns Hopkins University, who delivered the Willard Gibbs address on "Chemistry in relation to biology and medicine, with special reference to insulin and other hormones." With the modesty characteristic of really great men he related the history of chemistry in relation to life processes from Paracelsus to modern times, including a brief story of epinephrin and other discoveries of his own, which have brought him international fame. The lecture closed with some of the recent work on isolation of crystalline insulin. The medal was awarded at the Willard Gibbs Banquet, held in Ida Noyes Hall, the presentation address being made by Dr. JULIUS STIEGLITZ, who reviewed in a masterly fashion the scope and magnitude of the work which led to the conferring of this signal honor upon Professor ABEL. The whole meeting was featured by a fine spirit of cordiality, and it will be long remembered by every one who was fortunate enough to be present at the meeting.

The First International Congress of Soil Science.—Students of soil science and related sciences from all over the world met at Washington, D. C., June 13-22, in the first International Congress of Soil Science. Delegates were present from 39 different nations, representing practically all of the important agricultural states of the world.

The meetings were held in the spacious and beautiful halls of the United States Chamber of Commerce Building. The Congress was opened with an address by President COOLIDGE on Monday afternoon, June 13, following which there was a brief response from Dr. J. G. LIPMAN, president of the International Society of Soil Science.

After the first day the morning sessions were devoted to general programs, and the afternoon sessions to the specific work of the various commissions. These meetings of the six commissions were all very valuable, and an account of those of greatest interest to plant physiologists will be presented in the October number of *PLANT PHYSIOLOGY*.

To relieve the strain of prolonged discussion and presentation of new problems and data, the committee on arrangements provided for a number of field trips and social events, which were much enjoyed. The fourth day, for instance, was taken for a trip by motor bus into Western Maryland and the Shenandoah Valley of Virginia. Other excursions to Baltimore and Mount Vernon added to the attractiveness of the Congress. The trip on the Potomac to Mount Vernon was marred by the fact that the boat was of too deep draught to permit landing, but the river trip in itself was delightful.

Plant physiologists were well represented among those attending the Congress, and many features of the programs were of vital interest to them. The success of the Congress augurs well for future meetings of an international character. The next Congress will convene in Russia, which is only a just recognition of the great part Russian scientists have taken in the development of the science of Pedology.

The Fifth National Colloid Symposium.—The fifth National Colloid Symposium held at the University of Michigan, Ann Arbor, Michigan, June 22-24, 1927, was perhaps the most worth-while meeting since the second symposium in 1924. There was a genuine enthusiasm and considerable spirited discussion, with differences of opinion quite out of the ordinary. There were 250 registered as in attendance, besides a large number of local visitors.

Professor H. R. KRUYT, of the University of Utrecht, was the guest of honor. His paper on "Unity in the theory of Colloids" was greatly appreciated, and the discussion which followed lasted nearly all the morning session and showed the keen interest which his address aroused. Professor KRUYT captivated his audience with his wide knowledge of the subject, his keen wit and ready repartee. He remained at the University of Michigan after the meeting to deliver a series of lectures during the summer session.

The committee on entertainment arranged for auto trips about town and the neighboring countryside; golf at Barton Hills Country Club; a play on Wednesday evening, and the banquet on Thursday evening. A registration fee of \$3.00 covered these features, as well as the requirements for admission to the meetings. There were seven papers out of about 25 on the program that were of special interest to plant physiologists. It was re-

gretted by all that DR. LEONOR MICHAELIS was unable to attend the meeting and give his paper on "Investigations on Molecular Sieve Membranes."

The Nashville Meeting.—The meeting of the American Society of Plant Physiologists at Nashville, Tennessee, in December will be the fourth annual meeting of the Society. It should be the largest and most significant meeting so far held by this organization. All members are urged to respond promptly to the requests of the program committee for titles and abstracts of papers to be presented before the sessions at Nashville.

The program committee for this meeting consists of the following members of the Society: Dr. A. L. BAKKE, Iowa State College, chairman; Dr. C. R. BALL, U. S. Department of Agriculture; Dr. W. A. GARDNER, Alabama Polytechnic Institute; Dr. D. R. HOAGLAND, University of California, and Dr. S. V. EATON, University of Chicago. Cooperation with this committee, it is hoped, will be hearty and loyal. The committee invites suggestions which may assist them in planning for a highly profitable meeting.

Stephen Hales.—A short time ago Corpus Christi College, Cambridge University, celebrated the 250th anniversary of the birth (1677) of STEPHEN HALES. This event should focus the attention of plant physiologists on the early history of plant physiology, and the part that STEPHEN HALES played in the development of this branch of botanical science after the Renaissance. His book, *Vegetable Staticks*, published in February, 1727, just a month before the death of Newton, stands as a great landmark in the early eighteenth century; it was the first attempt at a systematic experimental study of an important physiological process, and may justly be considered the chief cornerstone of the historical foundation of vegetable physiology. HALES was 10 years old at the time NEWTON's *Principia* appeared, and it is quite certain that his association with NEWTON and other great leaders in the Royal Society of London inspired him to become the versatile churchman and scientist that we know him to have been. He was not only a Fellow of the Royal Society, but also "Rector of Farringdon, Hampshire, and Minister of Teddington, Middlesex." The book is "An account of some Statical Experiments on the Sap in Vegetables: Being an essay towards a Natural History of Vegetation. Also, a Specimen of an Attempt to Analyse the Air, by a great Variety of Chymio-Statical Experiments." The experiments were largely devoted to a study of sap pressures (by means of manometers), transpiration, and sap flow.

HALES was not only interested in sap pressure, but studied blood pressure in animals also, and won a permanent place in the history of animal and

human physiology by his studies in that field. He was also an ardent student of ventilation problems, and solved the problem of ventilating ship holds so as to prevent asphyxiation from accumulating fixed air, carbon dioxide. He also invented ventilators for preserving grains from molding, particularly corn.

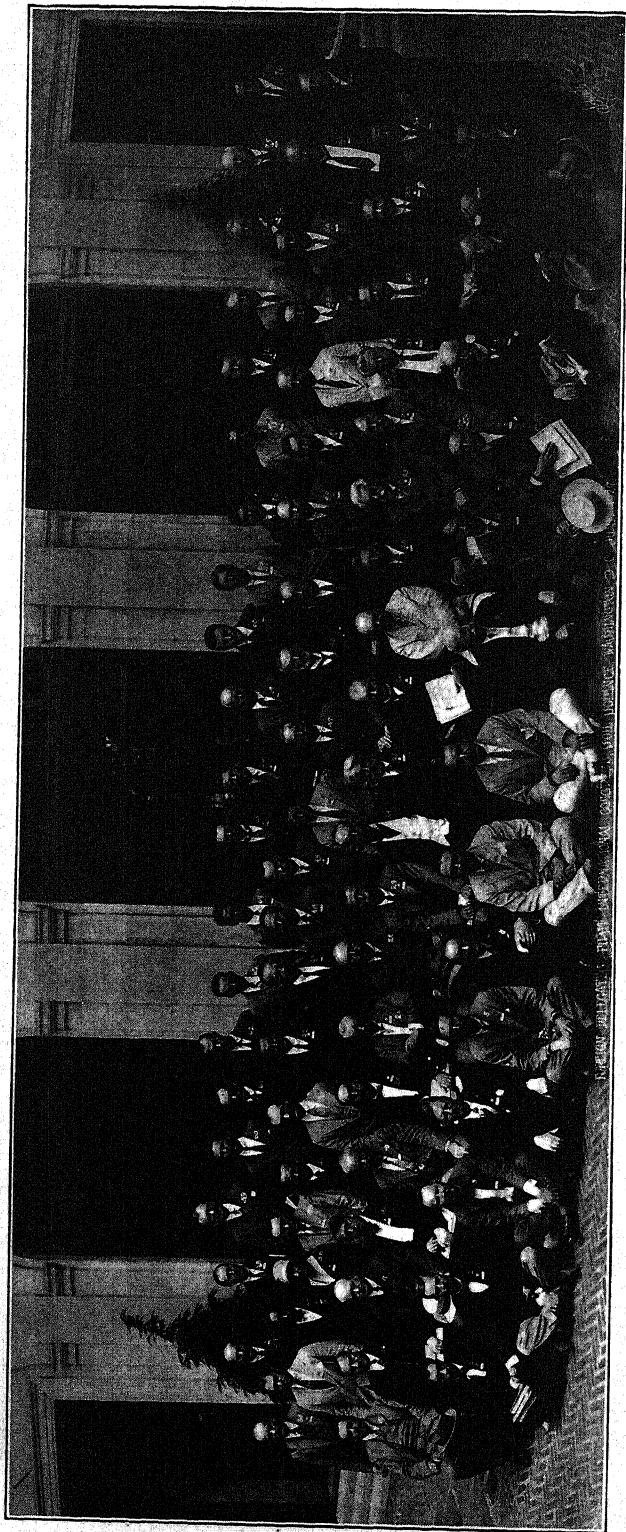
It is an appropriate time to remember HALES, and plant physiologists are proud of the fact that the chief cornerstone in the development of plant physiology, laid just 200 years ago, bears the inscription, "HALES, 1727."

Reports of the Committee on Analytical Methods.—This important committee has published a number of reports during the last two years. Attention is called to the fact that the chairman of the committee, Dr. W. E. TOTTINGHAM, the University of Wisconsin, has made arrangements to supply copies of these reports at a nominal price. The citations to the literature on methods are worth much more than the cost of these separates. Any one desiring to obtain copies of the reports should address the chairman of the committee.

Free Reprints.—Beginning with the year 1928, it is hoped that PLANT PHYSIOLOGY may be able to present a small number of reprints free to each author, provided the author has placed an order for reprints against which the first costs of making the reprints may be charged. The financial support of the journal has been such as to warrant the attempt to give authors some reprints without covers free of charge along with orders. As the support increases, the policy with reference to reprints can be made more liberal.

Plant Autographs and their Revelations.—A popular edition of J. C. BOSE's discoveries in the field of plant responses appears under this title from the press of the Macmillan Co. The characteristic point of view of the author is revealed in the preface where in speaking of the plant, he says: "In order to reveal the intricate mechanism of its life, it is necessary to gain access to the smallest unit of life, the 'life-atom,' and record its throbbing pulsation." Twenty-seven chapters and an appendix tell a marvelous story of sleeping, waking, fatigued, weeping, and nervous plants. If the book makes no more impression upon the lay public than BOSE's previous more serious scientific contributions have made upon scientific men, the book will do little harm. It is unfortunate that such books inevitably sow the seeds of misconceptions of plant life that require decades of effort to eliminate from the minds of uncritical readers. The publisher's price for this popularized edition of BOSE is \$2.50.

International Critical Tables.—The second volume of this monumental work has appeared from the McGraw-Hill Book Co. A number of the sections are of interest to biologists. The first section deals with the strength and related properties of woods, from North American, British, Danish, Dutch East Indian, Japanese and Asiatic, Mexican, South American, and West Indian sources. Farther on one finds a section on the durability, chemically, of glasses, which will interest analysts and other users of glass apparatus. Another section deals with animal and vegetable oils, fats, and waxes. Adhesives and gelatins; textile fibers; tanning and vegetable tanning materials; rubber, gutta percha, and balata; artificial plastics, such as nitrocellulose derivatives, and phenol resins and their products; raw materials of the paint and varnish industries; air conditioning; saccharimetry, and X-ray diffraction data are included in this volume, along with much other material of interest mainly to the industries. The largest single section deals with the metals and their alloys, pp. 358–610 being devoted to metallurgy. The volume contains 616 pages, and represents an enormous amount of work on the part of the compilers and editors.



FOREIGN DELEGATES TO THE FIRST INTERNATIONAL CONGRESS OF SOIL SCIENCE

1. AUGUSTO BONAZZI, ITALY
2. H. J. PAGE, ENGLAND
3. J. MIRTOFF, RUSSIA
4. G. W. ROBINSON, ENGLAND
5. B. A. KEEN, ENGLAND
6. A. A. J. DE SIGMOND, HUNGARY
7. A. L. DE KREYBIG, HUNGARY
8. CHARLES TELCKI, HUNGARY
9. PETER TREITZ, HUNGARY
10. A. SHOORIGIN, RUSSIA
11. H. M. NAGAN, CANADA
12. FRANK T. SHUTT, CANADA
13. THOS. RIGG, NEW ZEALAND
14. CHARLES A. FONTAINE, CANADA
15. H. HESSELMAN, SWEDEN
16. N. H. NIKLAS, GERMANY
17. B. SCHUSTER, GERMANY
18. PAUL KRISCHE, GERMANY
19. R. ALBERT, GERMANY
20. E. ABAD, SPAIN
21. V. C. MADSEN, DENMARK
22. S. MIKLASHEVSKI, POLAND
23. J. E. RUSSELL, ENGLAND
24. D. J. HISSINK, HOLLAND
25. K. D. GLINKA, RUSSIA
26. A. PENCK, GERMANY
27. N. STREMME, DANZIG
28. B. POLYNOV, RUSSIA
29. A. JARILOV, RUSSIA
30. L. T. PRASSLOV, RUSSIA
31. S. P. KRAVKOV, RUSSIA
32. S. A. SACHAROV, RUSSIA
33. MAHMOUD ABAZA, EGYPT
34. T. IMASEKI, JAPAN
35. ELIAS MELIN, SWEDEN
36. HUGO OSWALD, SWEDEN
37. P. G. KRISHNA, INDIA
38. JADURIGO ZIEMIECKA, POLAND
39. M. S. GORSKI, POLAND
40. F. TERLIKOWSKI, POLAND
41. R. MAC EAGHEN, URUGUAY
42. A. HAUSHOFER, GERMANY
43. C. NIKIFOROFF, U. S. A.
44. VICTOR HOHENSTEIN, GERMANY
45. P. P. JURIN, RUSSIA
46. N. M. TULAIKOV, RUSSIA
47. W. S. MARTIN, UGANDA
48. T. SAIDEL, ROUMANIA
49. M. DRACES, ROUMANIA
50. N. FLOROV, ROUMANIA
51. J. G. BIJL, HOLLAND
52. A. SOLOLOVSKI, RUSSIA
53. A. A. SCHMUCK, RUSSIA
54. W. W. GEMMERLING, RUSSIA
55. G. WIEGNER, SWITZERLAND
56. L. F. SMOLIK, CZECHOSLOVAKIA
57. MEIR WINNIK, PALESTINE
58. A. J. FINDLAY, NIGERIA
59. C. H. KNOWLES, GOLD COAST
60. F. HARDY, TRINIDAD
61. C. L. WHITTLES, BRITISH GUANO
62. ADOLPH REIFENBERG, PALESTINE
63. HANS JENNY, SWITZERLAND
64. A. B. FAGUNDES, BRAZIL
65. A. B. CATLEY, AUSTRALIA
66. H. W. KERR, AUSTRALIA
67. J. W. TURIN, RUSSIA
68. E. E. USPENSKI, RUSSIA
69. E. M. CROWTHER, ENGLAND
70. K. SHIBUYA, JAPAN
71. LOPEZ DOMINGUEZ, PORTO RICO
72. V. NOVAK, CZECHOSLOVAKIA
73. F. MENCHIKOWSKI, PALESTINE
74. C. T. GIRSBERGER, SWITZERLAND
75. F. SCHUCHT, GERMANY
76. H. GESSNER, SWITZERLAND

PLANT PHYSIOLOGY

OCTOBER, 1927

THE FIRST INTERNATIONAL CONGRESS OF SOIL SCIENCE

CHARLES A. SHULL AND FRANK THONE

The first International Congress of Soil Science, which was held at Washington, D C., last June, was an important event in the development of soil science. After a number of international conferences beginning in 1909 at Budapest, and ending at Rome in 1924, it was decided to hold this world-wide meeting, which should bring together the ablest investigators of soil phenomena from all of the great agricultural nations. The congress was organized under the auspices of the International Society of Soil Science, the American Society of Agronomy, and the United States Department of Agriculture, and proved to be a very successful and profitable meeting. The leaders of thought in this field were assembled in congress to share their knowledge and points of view, to take stock of their progress, to develop concerted attack upon the unsolved problems, and to gather inspiration and enthusiasm for the future development of soil science. The meeting had one other happy effect. It centered the attention of the public at least temporarily upon the importance of the advances made in our knowledge of the soil, and the even greater importance of further extension of our knowledge through fundamental research.

Nearly 500 delegates and visitors were in attendance at the meetings of the congress. Representatives were present from Australia, New Zealand, India, Egypt, Palestine, Japan, British East, South, and West Africa, Germany, Russia, England, Italy, Norway, Sweden, Denmark, Holland, Switzerland, Mexico, Brazil, Chile, Uruguay, the Central American republics, and many other countries. The United States and Canada had more than three hundred delegates and visitors participating in the congress. The frontispiece of this number shows a group of the foreign delegates, and the names in the key correspond to the numbers in the photograph, reading from left to right, and from below up.

The congress convened for its first meeting on the afternoon of June 13, in the beautiful building of the United States Chamber of Commerce. The meeting was opened by an address by Hon. CALVIN COOLIDGE, President of the United States, who stressed the importance of the soil in human affairs. The response was by Dr. J. G. LIPMAN, president of the International Society of Soil Science. The work of the congress began in earnest on Tuesday, June 14. The presidents of the several commissions made brief reports of progress in the work of their respective commissions, after which President LIPMAN gave an address on Soils and Men. There was also an address by CHARLES H. McDOWELL on Fertilizers and Soil Science. The afternoon sessions were more technical in nature, and were held under the auspices of the several commissions which make up the congress. The work was organized under six commissions as follows: The first commission concerns itself with soil mechanics and physics, mechanical analysis, etc.; the second with soil chemistry. The third considers soil bacteriology and biochemistry; the fourth soil fertility; the fifth deals with nomenclature, classification and mapping of soils, and the sixth commission handles the problems of application of soil science to land cultivation. It is evident that the work of commissions two, three, and four are of greatest interest to plant physiologists; this report deals particularly with some of the papers presented before these three commissions, and with some of the addresses at general sessions.

Commissions II and IV met together, with Dr. A. A. J. DE SIGMOND, of Hungary, presiding. The meeting opened with a discussion of methods of chemical determination of nutritive materials in soils by DE SIGMOND, who proposed a cooperative study of soils in many different countries under the auspices of the second, third and fourth commissions. The plan called for the testing of a large number of soil samples in each region in accordance with a standardized procedure, with attention paid to both soil and subsoil.

A number of papers concerned themselves with the problems of exchangeable bases in the soil. Dr. J. S. JOFFE and H. C. McLEAN, of the New Jersey Station, tested the availability of replaceable cations in unfertilized soils. The soils were treated with neutral salt solutions, such as CaCl_2 , MgCl_2 , KCl , NaCl , and NH_4Cl , until all replaceable cations of the soil had been removed by exchange, after which the soils were washed with purified distilled water till all chloride ion was removed. In other cases 0.05 N. phosphoric acid was used to replace cations. These soils were used in sand cultures as sources of N, P, K, Ca, Mg, and Na to test the availability of the cations of the soil for growth of buckwheat. The results indicate that plants are capable of using the cations of the soil, even if they are tied up chemically in the complex.

Dr. H. J. PAGE, of the Rothamsted Station, discussed the relation between the state of saturation of the soil and its H-ion concentration, with special reference to the action of "physiologically acid" fertilizers. He found that as the acidity of the soil increases, the exchangeable base decreases. At a reaction value of pH 4.04 there was only one seventh as much exchangeable base as at pH 5.22. The production of acidity by $(\text{NH}_4)_2\text{SO}_4$ was discussed. While this salt does acidify the soil, the acidity is not caused as is usually thought, by greater use of NH_4 than of SO_4 by the plant. In the presence of the soil complex, the NH_4 ion is believed to replace Ca ion, with the formation of CaSO_4 . This change does not lead to acidity. There is an indirect chemical change following the exchange of NH_4 for Ca, in which part of the ammonia nitrogen is oxidized to HNO_3 . The acidity, then, arises indirectly by chemical action from the NH_4 ion.

Novel theories of plant nutrition were presented by Dr. EMIL TRUOG, of the University of Wisconsin, who believes that plants directly attack the minerals of the soil. He relates the root hair to the feeding of the plant, and illustrates the idea by analogy to a collodion sac containing acid, in contact with a piece of calcite. He distinguishes "solid phase" feeding from "liquid phase" feeding. The water of the soil-root hair region has a pH of about 4.0 when saturated with CO_2 , and as carbonic acid is the only root excretion normally, it is held that solid phase feeding is greatly increased by the output of CO_2 from the root. Buckwheat and sweet clover are strong feeders on raw rock phosphate, while oats and corn are weak feeders on this mineral. This difference is related to the ability of the plants to feed on the solid phases, the plants not being dependent on the soil solution for their minerals.

The significance of traces of certain inorganic elements of the soil for plant growth was emphasized by Dr. J. S. McHARGUE, of the Kentucky Station, who has given much attention to such elements as manganese, copper, zinc, nickel, cobalt, barium, strontium, boron, arsenic, fluorine, bromine, iodine, etc. These elements occur widely distributed in igneous and sedimentary rocks, and in soils derived from them. Plants grown on the soil take in some of these elements and may deposit them more abundantly in just certain tissues, as in the germ, or pericarp, or young tender parts of the plant. Manganese and copper are particularly needed by plants, and chlorosis is produced when they are excluded. Such chlorotic plants contain from one half to one tenth of the normal amount of manganese. When treated with gum guaiac and H_2O_2 , the chlorotic leaves give a poor test for peroxidase, while those given the normal amount of manganese give a strong peroxidase reaction. These observations support the view that manganese is related to the oxidizing system of the plant. The

iron content in these cases was as large in the chlorotic as in the non-chlorotic leaves, so that manganese seems to be related to the synthesis of chlorophyll, also.

Some of the analyses reported were very interesting. Expressing the elements in milligrams per kilogram of dry weight, McHARGUE found in wheat bran 16 mg. copper, 125 mg. of manganese, and 75 mg. of zinc. The germs of wheat contained 46, 150, and 160 mg. of these elements respectively, while flour made from wheat in which germs and bran were removed contained only a trace of copper, 10 mg. of manganese, and 22 mg. of zinc per kilo of flour. Cotton seed kernels contained 54 mg. copper, 13 mg. manganese, and 320 mg. zinc. Oats contained 30, 58, and 89 mg. of the three elements, respectively.

The fact that marine animals and domesticated animals both contain manganese, copper, and zinc in appreciable quantities, especially in the embryos, and the glandular organs, liver, kidneys, spleen, pancreas, and such vital organs as heart and brain suggests their importance in animal metabolism, just as in plant metabolism. Part of the superiority of the stock raised in the blue-grass region of Kentucky is traced to the soil, which is derived from a phosphatic limestone, rich in manganese, copper, zinc, nickel, and cobalt. These elements which occur merely as traces in plant ash, are surely of greater importance than has usually been stated in physiological reference works.

A rapid method of determining the sulphur deficiency of soils was outlined by Dr. F. J. ALWAY, of the University of Minnesota, who uses the total sulphur content of alfalfa hay grown on the soil as his criterion. When the alfalfa contains 0.09–0.11 per cent. of sulphur, applications of gypsum give fine increases in crop yields, up to 70 per cent. increase. When the analyses show 0.12–0.15 per cent. of sulphur in the hay, there is a slight response from application of sulphur carriers. In crops where the sulphur content runs from 0.26 to 0.50 per cent., there is no response from applications of sulphur fertilizers. It is best to use only leaves in the analyses, as these contain more sulphur than the stems, and are affected more by sulphur additions to the soil than stems are. If one secures samples of alfalfa from all over a given region, and analyzes the samples for sulphur, one can tell very shortly where all the sulphur deficient land areas lie, and tests can be run on these to determine whether the increased crop yields will pay for the cost of application of sulphur fertilizers.

The influence of phosphorus in hastening maturity was emphasized by Dr. J. J. SKINNER, with reference to cotton. Cotton fertilized with phosphate matured 69 per cent. of its total yield by September 7, while untreated cotton matured only 47 per cent. of its yield by that date. It requires from

70 to 90 pounds of phosphate per acre to produce this effect. None of the other fertilizer ingredients produced as much effect as phosphorus in this regard.

The general session on June 15 was marked by several splendid addresses. The outstanding feature of the meeting was the discussion of the present status of soil biology, by Sir JOHN RUSSELL, Director of the Rothamsted Experimental Station. He considered the relation of the reaction of the soil to living organisms, protozoa, bacteria, algae, fungi, actinomyces, nematodes and other worms, snails, etc., and showed that the flora tends to become fungal on acid soils. Aeration is also an important factor determining the microbial life of the soil. Changes in the flora and fauna can be followed under various conditions by cultural isolation, microscopic examination, and population counts. The level of numbers of organisms present depends on the amount of energy available in the humus jelly which coats the soil particles. An increase in energy supply causes increased numbers of organisms to develop. Addition of a single substance may favor just one group, but addition of complex organic matter will favor many groups of organisms, providing not only a larger number, but also a larger variety in the soil life.

Referring to his own work on bacteria and protozoa, he gave an account of the fluctuations in number of these organisms from day to day and from hour to hour. The periodicity is related to the reproductive activities and feeding habits of the respective organisms. The amoebae feed upon the bacteria and increase as bacteria decrease, but later the bacteria again increase and the amoebae die off. In other cases of fluctuation the causes are more complex. *Bacillus radicola* fluctuates when it is the only organism present. In some cases the fluctuations may be related to different modes of reproduction in different parts of the life cycle.

He discussed the modifications produced in soils by sterilization, and finds that the best way to avoid serious modifications is to dry the soil *in vacuo*. Poisonous decomposition products came in for consideration, and the practical applications of all these studies were stressed. The more active the soil population, the more fertile the soil; and activity may be increased by feeding the population appropriately. Addition of sugar for increased activity has been tried in some places.

The new methods of nitrogen fixation were referred to as making nitrogen fertilizer cheap. The oxidation of sulphur and control of the reaction of the soil were discussed in some detail, and some of the newer methods of making manures from straw were described. Cellulose can be converted into useful manure by moistening, adding nitrogen compounds and phosphates, and allowing organisms to bring about partial decay in the mass.

The address closed with a discussion of the value of soil microbiology as a part of the education of the country boy and girl.

In the same meeting Dr. O. LEMMERMANN, of the Agricultural Hochschule, Berlin, discussed the determination of soil acidity, Dr. A. ITANO, of the Ohara Agricultural Institute, Japan, gave an account of the present status of soil investigations in Japan, and Dr. A. F. WOODS, director of research in the Department of Agriculture, described the origin and the objects of the Bureau of Soils.

The session for reading of technical papers Wednesday afternoon contained many helpful papers. Dr. JOHN S. BURD, University of California, discussed some of the problems of soil solution research. Careful study of displaced solutions and water extracts of soils reveals decreasing concentration of nutrient ions with advance of the crop season, and secular decline after a few years of cropping, accompanied by decreased production. Phosphate ion and potassium ion are the main ones involved in these deficiencies. While phosphate ion is more abundant in arid than in humid soils, the drier soils are more highly buffered against acidity than humid soils, and this partly nullifies the more favorable PO_4 concentration. Temporary acidity developed at phase boundaries in soils containing calcium carbonate sets free Ca which also tends to depress phosphate concentration.

The potassium relations are more difficult to explain. In some cases additions of potassium will increase the concentration of K-ions long before the replaceable base complex has become saturated. Such soils either need no fertilizer, or may be profitably amended. In other cases, however, the soils have such extraordinary "fixing power" for potassium that there is no economic way of increasing the potash content of the soil solution. This is especially true if the soil is at the same time highly buffered against acidity.

The phosphorus content of soils was discussed by Dr. F. W. PARKER, Alabama Polytechnic Institute, who used displaced solutions and 1:5 water extracts for his measurements. The phosphate content is often very low, the average for displaced solutions from 20 American soils being 0.09 parts per million for inorganic PO_4 , and 0.47 parts per million of organic PO_4 . These low amounts do not necessarily indicate infertility, for several soils that showed only 0.03 ppm. of the inorganic phosphate ion were still good productive soils.

Absorption studies with corn, soy beans and buckwheat showed that they absorb only the inorganic phosphate. In 6 to 24 hours all of the inorganic PO_4 would be removed from the solution by the roots; but the organic PO_4 was not absorbed in 24 to 48 hours. Maximum growth of corn occurred at 0.50 ppm. of the inorganic phosphate ion, and with 0.05, 0.10, and 0.25

ppm., the growth was 19, 37, and 71 per cent., respectively, of the maximum. The plants cannot reduce the PO_4 to much less than 0.03 ppm. The two outstanding fact here are that the phosphorus in displaced solutions is mainly organic phosphorus, and that water soluble organic phosphates are not available to plants.

The soil solution often has too little inorganic PO_4 in solution to be adequate. The plant must therefore have contact with solid surfaces in the soil. Excretion of CO_2 is thought not to increase PO_4 solution, but the root is believed to dissolve the rock at points of contact.

Dr. D. R. HOAGLAND discussed the potassium relations in soils, and the relative value of adsorbed and soluble K. He raised the question whether all of the replaceable K in the soil has equal physiological value. Non-zeolitic minerals assist in the maintenance of available K in the soil. All such work must be considered in reference to the plant, which is concerned with all types of soil minerals. It is very desirable, and necessary, to correlate soil chemistry and plant physiology.

The effects of 14 years of potash manuring upon both plants and soils were presented by Dr. H. NIKLAS, of the Weißenstephan Agricultural Institute, Germany. There was no change in the pH values of the soil from the fertilizer, but microorganisms increased. The molds were not depressed, but there was less nitrogen fixation where K fertilizers were applied alone. No permanently unfavorable physical conditions were developed in the soil. Different treatments gave different amounts of dispersity of soil particles, and the greatest dispersion was caused by use of KCl. When KHSO_4 was used along with some other potash salts, a condition intermediate between gel and dispersed state was developed. The effects of such changes on aeration and drainage were mentioned.

The growth of root hairs of cabbage was reported upon by Dr. C. H. FARR, Washington University, who kept the root hairs in flowing solutions while under the compound microscope. The growth was recorded at 10-minute intervals for 3 hours. A unique three-dimensional graph was constructed to represent the growth, with the rate of elongation as the ordinates, and the pH value and concentration of CaCl_2 as the two abscissae. There was a wide range of pH for growth, from 3.4 to 11.9, but there was no growth in hydrochloric acid alone, nor in the more acid solutions of low CaCl_2 content. Acid solutions with a moderate supply of calcium gave fair growth, but the best growth was in a nearly neutral solution with only 0.02 M CaCl_2 present. However, the growth in $\text{Ca}(\text{OH})_2$ solutions at pH 9.9, and in 0.008 M CaCl_2 at 7.9 was almost as good as in the neutral solution.

In the general session of Friday morning two very striking papers were presented. One was an illuminating study of the trend of land utilization

in the United States by Dr. O. E. BAKER, of the Department of Agriculture. Previous to 1900, migration was from poorer to better lands. Railroad development made possible the conquering of the prairies during the last half of the nineteenth century. Since 1900 the movement has been onto poorer lands, since all the good land was in use. By 1910 the brown lands had been occupied, and by 1920 the cycle of spread had ended. The entire country was settled. While land use has declined since 1920, production has increased 14 per cent., which is 60 per cent. greater than the increase in population. The causes of this situation are not related to the soil, but to efficiency of unit production. Less productive animals give way to more productive ones, and so we find milk production up 25 per cent.; beef production, 7-8 per cent.; pork and lard, 17 per cent.; chickens, 15 per cent.; and eggs, 16 per cent. The change from horse power to traction has been a contributing influence. Such changes as this are bound to continue until an equilibrium between agriculture and other industry is reached.

The other paper was by Dr. ALBRECHT PENCK, University of Berlin, on the productive capacity of the globe. He pictured the world with maps in terms of calorie production, and claims that the number of people that can live on the earth is calculable on the basis of the amount of food that can be grown, and the calorie requirement per individual. In the century from 1820 to 1920 the population of the earth doubled, and if 2,500 millions were all that the earth could feed, we should be overpopulated sometime in this century. But PENCK calculates that 8,000 millions may finally inhabit the earth. Each continent would have a definite density based on its capacity to produce food. The greatest density is possible in the moist tropics. At present the population is sparse in those regions, but a gradual penetration and acclimatization will take place. He makes Brazil ultimately the most populous country in the world. This is, of course, only a provisional calculation.

At the same meeting the chemical characteristics of soil leachings were discussed by DE SIGMOND, and a paper by GLINKA on the history of Russian soil science was read by Dr. LIPMAN.

Perhaps the most challenging paper before Commission III, on soil bacteriology and chemistry, was that of Professor JULIUS STOKLASA, of the Technical Institute and Experiment Station, Prague. Due to illness, Professor STOKLASA was unfortunately not able to be present in person, but his paper was read before the commission.

Discussing the significance of bacteria in soil productivity, Prof. STOKLASA departed most decidedly from a number of notions about the part played by microorganisms, ideas hitherto accepted as orthodox. Perhaps the most important rôle they play, in his opinion, is the production of increased

quantities of carbon dioxide and its release into the lower strata of air, where it may be captured by photosynthetic organs. He is also convinced that carbon dioxide enters plants in solution through the roots.

Carbon dioxide evolution proceeds more rapidly from fertile soils than from unproductive ones. His experiments have shown a production of 60 to 120 mg. from one kg. of fertile soil, as against production of 15 to 20 mg. from the same weight of an unfertile soil.

A further rôle of bacteria, he said, is their activity in rendering available for the higher plants insoluble or partially insoluble mineral salts by chemically altering them into soluble compounds. A large part of the fertilizer increment must be regarded as food for the bacteria themselves: "I see the value of the addition of nitrogen, phosphorus and potassium to the soil in the increase of energy due to the multiplication of the microorganisms, and in the stimulation of the metabolic processes of the heterotrophs, resulting in an increase of the dissimilation processes and production of carbon dioxide and organic acids."

The whole problem of CO_2 relations is a debatable one. Dr. LEMMER-MANN of the Agricultural Institute, Berlin-Dahlem, disagreed with STOKLASA regarding the importance of soil respiration in the carbon dioxide economy of the higher plants. Although considerable quantities of this gas are evolved, he said, air circulation quickly removes it from the air strata where it might be of benefit to the plants.

On the other hand, experiments reported by Dr. H. LUNDEGARDH, of the Stockholm Experimentalfältet, tended to support the contentions of STOKLASA. In greenhouse experiments it was found that an increase of carbon dioxide in the atmosphere always produced a corresponding increase in yield. The ratio of assimilation to CO_2 concentration was found to be more favorable at 30° than at 20° or 10° C. "The CO_2 evolution is due to the activity of microorganisms, and is an indicator of the total metabolism and of the fertility of the soil. Most of the field experiments have shown a distinct parallelism between the CO_2 factor and the crop yield."

The quantitative aspects of soil bacteriology were discussed by a number of speakers, among them Dr. S. WINOGRADSKY, of the Pasteur Institute, Paris; Dr. G. ROSSI and S. RICCARDO, Royal Superior Agricultural Institute in Portici, Italy; Dr. J. K. WILSON, Cornell University; Dr. A. G. LOCHHEAD, Central Experimental Farm, Ottawa, Canada; and Dr. H. J. CONN, of the New York Agricultural Experimental Station. The complexity of the problem and especially the inadaptability of the standard methods of microbiology received special emphasis. Standardization of methods is much needed.

The present position of our knowledge of the distribution and functions of algae in the soil was discussed by B. MURIEL BRISTOL-ROACH of the Roth-

amsted Station. The author stated that many of these organisms are capable of growing saprophytically in the dark, provided a suitable supply of organic food is present.

Second only in importance to the bacteria in the soil flora, if indeed second to them, are the fungi. The parts they play were discussed by Dr. CHARLES THOM, of the United States Department of Agriculture, WILLIAM B. BRIERLEY, Rothamsted Experimental Station, and Dr. J. MAGROU, of the Pasteur Institute, Paris. As with the soil bacteria, quantitative methods are as yet tentative, and standardization of methods is much needed. The study of fungus physiology, essential to an understanding in their activities in the soil, is complicated by the readiness with which they sporulate or pass into other resting or resistant phases, and by the polymorphism of both the active mycelia and spores.

Dr. MAGROU called attention to the shaken position of mycorrhiza as true symbionts. In many cases they have been proved to be harmful—genuine parasites; in others, the host plant, though not harmed by their presence, has shown itself well able to get along without them; and in still further cases the host, needing them during infancy, can dispense with them at maturity.

Atypical nitrogen-fixing activities, influenced by the highly acid conditions in the soils of Finland, formed the subject-matter of the paper by Dr. WIDAR BRENNER, of the Geological Institute of Helsingfors. *Azotobacter* plays no part here; its place is taken by associations of bacteria and fungi. Though the nature of these associations is as yet little understood, experiments have shown that they can fix nitrogen as efficiently as can *Azotobacter*.

The chemistry of free-living nitrogen-fixing bacteria was considered by Dr. C. STAPP, of the Biologische Reichsanstalt für Land- und Forstwirtschaft, Berlin. In this group the *Azotobacter* type is the most abundant and best known among obligate aerobes. *B. asterosporus* is given as typical of the facultative anaerobes, while among the obligate anaerobes *Amylobacter* is the leading example. The nodule bacteria, of course, are the principal representatives of the nitrogen-fixing symbiotic forms.

Virtually nothing reliable is known of the biochemistry of nitrogen fixation. "It is quite possible that in the gas-forming nitrogen-fixing organisms hydrogen *in statu nascendi* will join the atmospheric nitrogen within the bacterial body to form ammonia, and that from this product a high molecular weight nitrogen-containing substance (protein) originates. On the other hand, this can hardly be the case for the non-gasogenes." The author expresses no choice between the opinion (1) that a direct combination of carbon and nitrogen compounds results in the "bausteine" of pro-

teins, and (2) that a catalytic action, through the agency of enzymes (these as yet unknown) first gives rise to ammonium nitrite.

"It is as yet uncertain whether it will be possible to raise bacterial strains or races with a higher nitrogen-fixing power. An increase in 'virulence' of the nodule bacteria is said to be possible by repeated passage through plants. But the author has not been able to confirm these experiments by his own tests during the past year."

Finally, STAPP regards the practicability of inoculation of soils with *Azotobacter* or other free-living nitrogen-fixing organisms as very problematical.

Legumes are not the only higher plants that can secure nitrogen for themselves by cooperation with bacteria; nor need there be complete symbiosis, as in the case of nodule bacteria, to bring about this cooperation. GEORGES TRUFFAUT and N. BEZSSONOFF, of Versailles, France, described experiments with maize raised in media completely free from nitrogen for the plants, and deprived of all organic matter on which the bacteria might feed. The only possible source of nitrogen for either corn or bacteria was through fixation from the atmosphere by the bacteria, and the only possible source of energy for the bacteria was from the root secretions. Yet the maize thrived and grew to maturity.

Dr. E. B. FRED, of the University of Wisconsin, described his researches on the butyric-acid formers that live anaerobically in many soils. They are a very complex group, motile, spore-forming, non-pathogenic, clostridial-shaped; 46 strains belonging to two main subdivisions have been isolated. In general, they are much inferior in nitrogen-fixing powers to cultures of *Clostridium pasteurianum*.

KEIZO HIRAI and IWAO HINO, of Kyushu Imperial University, Fukuoka, Japan, reported their experiments on the influence of soil protozoa on the activities of *Azotobacter*. They found that the presence of the protozoa generally stimulated nitrogen fixation by the bacteria. They ascribe this to the tendency of the protozoa to render the medium alkaline, thus reducing the acids formed by *Azotobacter*, which in pure culture soon reach a concentration great enough to limit the growth of the organisms.

The nature of the organic matter of the soil, particularly its humus, was discussed by a number of participants, including H. J. PAGE, Rothamsted Station; SELMAN A. WAKSMAN, New Jersey Agricultural Experiment Station; G. W. ROBINSON, J. O. JONES, and R. J. EVANS, University College, Bangor, North Wales; OSWALD SCHREINER and P. R. DAWSON, United States Department of Agriculture, and EDMUND C. SHOREY, also of the Department of Agriculture.

A related subject, the bacterial decomposition of cellulose, also called forth a number of papers, among them contributions by Y. KHOUVINE,

Pasteur Institute, Paris, and R. J. DUBOS, New Jersey Agricultural Experiment Station. The taxonomy of these bacteria is very little known; only one, *B. cellulosa dissolvens*, has been isolated with certainty. Suitably cultured, it breaks cellulose, as filter paper, down into (1) a yellow-orange dye stuff, (2) acetic and butyric acids, (3) hydrogen and carbon dioxide, and (4) alcohol. The spores of this organism are highly resistant; they have been found alive in 25-year old soil samples.

Dr. LIPMAN contributed an important paper before this Commission, giving a general survey of the microbial aspects of green manuring. Plowing under green manures is not always and unqualifiedly good for the soil; much depends on the number and kinds of microorganisms present, and on the composition of the manures themselves. When the amount of organic matter is large and the conditions favorable for rapid decay the composition of the soil air may be profoundly modified. Large quantities of CO_2 are produced at the expense of the soil air. Oxidation and reduction processes in the soil solution are directly influenced thereby. No less far-reaching is the effect of green manures on the potential supply of nitrogen to crops. The fermentation following the plowing under of a green-manure crop may increase or decrease the amount of so-called available nitrogen. Green-manure crops relatively rich in carbohydrates may cause the nearly complete disappearance of nitrates, and may later depress their accumulation in the soil solution. On the other hand, green manures containing a relatively high proportion of amino-compounds and proteins may exert an early and markedly favorable influence on the formation of ammonia and nitrates.

The fermentation of green manures involves other important changes. There is a temporary modification of the soil reaction, and a passing tendency toward an increase in the hydrogen-ion concentration of the soil solution. Substances toxic to young plants may develop; the proportion and quantity of mineral nutrients may be modified and the complexion of the microbial flora greatly changed. By controlling these changes an optimum microbial balance for the growth of higher plants may be maintained.

RUSSELL contributed a paper summarizing the present status of soil biology and its bearing upon agricultural practice. The relationship between soil biology and agriculture is in part that the soil microorganisms are the chief agents bringing about the changes in the soil organic matter, and in part that soil microorganisms have certain direct effects on growing plants.

The changes in organic matter that affect soil fertility are of two kinds: first, the addition of organic matter by fixation of CO_2 by algae, of gaseous

nitrogen by *Azotobacter*, *Clostridium*, etc., and of ammonia and nitrates by a large variety of organisms; second, the decomposition of organic matter in the soil, resulting in (a) the disintegration of the structural material of the cells and of dead plant residues with consequent destruction of the fibrous material which in certain circumstances exerts a harmful physical effect upon the soil, (b) the formation of colloidal organic substances which are normally advantageous to the soil, (c) the formation from complex organic compounds, unsuited to plant nutrition, of simple organic compounds of nitrogen, phosphorus and sulphur entirely appropriate to plant nutrition, and (d) the destruction of compounds formed during the above changes that are harmful to plant growth.

A brief review of the present knowledge of symbiosis and parasitism was given by the author. Although soil microbiology is a comparatively new science, it has already had four clear-cut and important applications: the inoculation of leguminous crops, the partial sterilization of soils used for horticultural purposes, the manufacture of a useful fertilizer from sewage, and the decomposition of cellulosic materials with production of a humus manure closely resembling farmyard manure.

Other features of the congress are worthy of mention. The opportunities for social contacts were delightful. Receptions, dinners, and excursions interspersed among the meetings prevented any monotony from the scientific programs. Thursday was devoted to a motor bus excursion into western Maryland and the Shenandoah Valley of Virginia. On Saturday afternoon there was a boat excursion down the Potomac to Mount Vernon, which unfortunately did not permit the excursionists to land; on Tuesday following, an excursion to Baltimore, with dinner on return at the University of Maryland. The next day, June 22, saw the beginning of the trans-continental excursion, and the close of the formal sessions. These social events put everyone at ease, and created a friendly atmosphere that pervaded the entire congress.

This account of the meeting should not close without reference to the general exhibition which was held in the rooms adjoining the meeting places. Several of the foreign nations sent splendid exhibits of their activities. Russia made a very favorable impression by its large and varied display. There were maps showing their soil surveys, periodicals, publications, text books, soil sections of typical Russian soils, portraits of the great Russian scientists who, more than any others have been responsible for the development of Pedology. It was a delight to see this galaxy of Russian stars—DOKUCHAIEV, GLINKA, GEDROIZ, JARILOV, KOSTYCHEV, BEKETOV, KOSSOVITCH, OMELIANSKY, BOGOSLOVSKY, SABININ, VERNADSKY—a group of whom any nation would be proud. The exhibit of books by

these leaders of thought and research was a revelation to those who examined them.

Latvia had also placed on exhibit 12 beautiful soil profiles, soil maps, and publications by J. WITYN. There were soil sections and maps from Roumania, showing the interest that is being taken in a careful study of the soil in many quarters of the world.

The Bureau of soils had placed on exhibit some of the activities of the Department of Agriculture. Maps were on display, and various bibliographies which have in recent years proved such a valuable part of the work of the Department of Agriculture Library. The list of publications on soils 1844-1926, and a classified list of soil publications of the United States and Canada are examples. A number of the best journals devoted to soil science were on exhibit and more than 50 recent volumes on various aspects of soil science. Some very early agricultural literary treasures had been entered, such as HUMPHREY DAVY's *Agricultural Chemistry*, 1819; photostatic copies of the *American Farmer*, 1820; EDMUND RUFFIN's *Essay on Calcareous Manures*; JOHN BINNS's *Treatise on Practical Farming*; RICHARD PETERS, *On Gypsum*, etc.

Some of the apparatus and individual exhibits were of more than usual interest. The Rothamsted Experimental Station had sent one of its dynamometers, along with isodyne maps and surface representations of the soil resistance to plowing. These were explained on occasion by Dr. KEEN who has developed these methods of studying variability in soil consistency, and the effects of treatments of the soil on plow draught. Apparatus for mechanical analysis of soils by the British Official Methods was shown.

Models of a nitrogen-fixing plant, a sewage disposal plant, the Cottrell precipitation for PO_4 recovery in the volatilization process, a flow diagram of the direct synthetic ammonia process, and a map of our potash resources represented some of the fertilizer activities. A group of pure organic compounds isolated from soils had been sent over from the Department of Agriculture.

Among the soil testing apparatus were found the apparatus for colloidal analysis according to BOUYOUKOS, soil centrifuges, baths, pyrex glassware, electrotitration and colorimetric devices for hydrogen ion determinations, Chamberland filters, percolation tubes, motor stirrers, specific gravity apparatus, microscopes, Kjeldahl apparatus, the Hutchins apparatus for the study of oxygen-supplying power of the soil, and many others. The Chambers-Wright micro-manipulator for single cell isolations was demonstrated daily.

Some experiments on plant nutrition were running in the exhibition room. Manganese deficiency was demonstrated, there was a guanadine

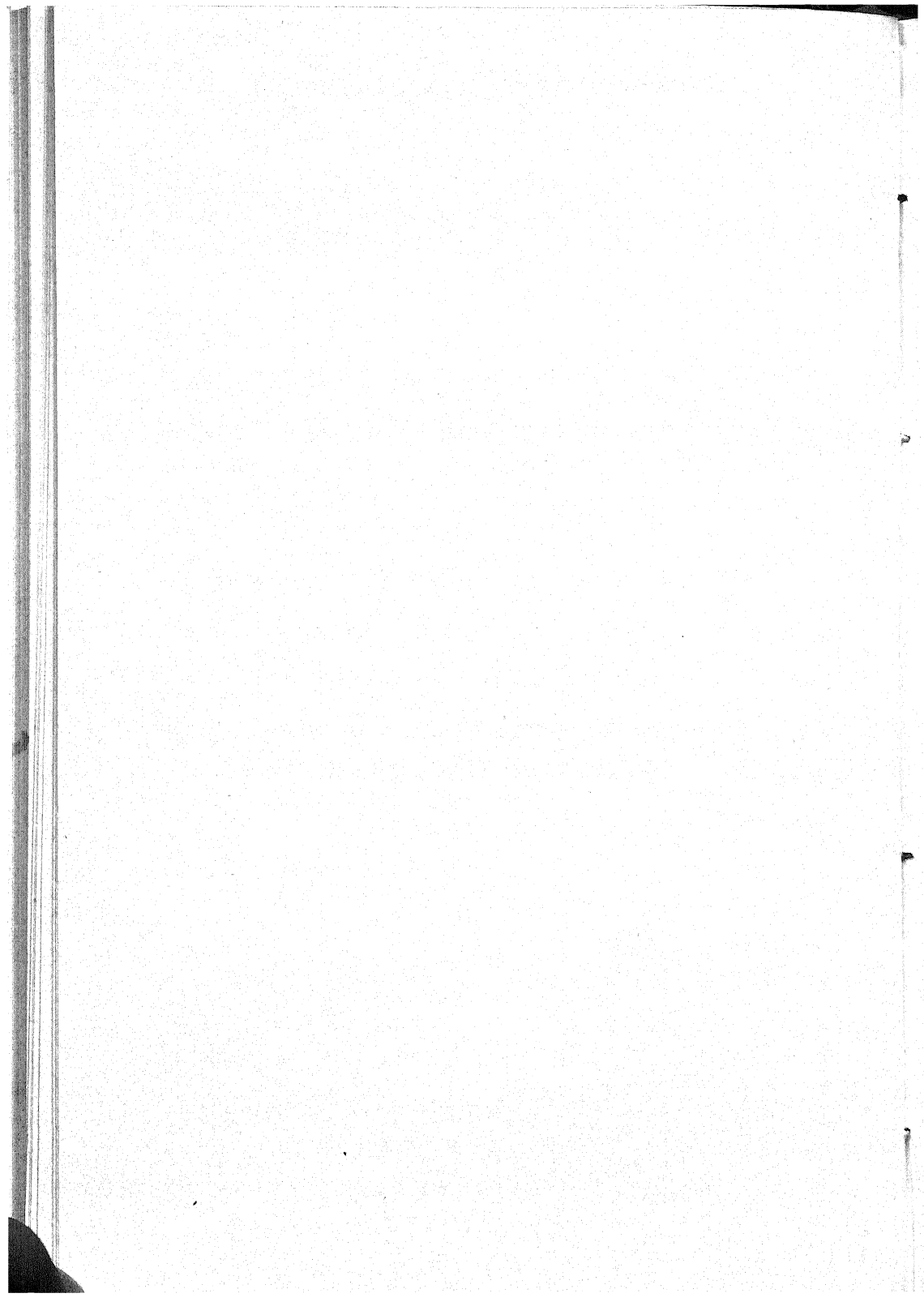
experiment showing toxicity, and a nucleic acid test showing its beneficial action. A soil fertility test for corn had been set up.

Two other features of the exhibition will close this account. A chart showing the decomposition of plant materials in the soil by microorganisms had been prepared. The substances started with, included 6 groups of soil constituents: water soluble, celluloses, pentosans, proteins, lignins, and the fats, waxes, and cutins. The products of breakdown of each class were listed, and in all but the last two, the important product was the microbial cells. The lignins leave undecomposed and unknown residues, as do also the fatty and waxy substances. But the humus of the soil is made up almost entirely of the remains of microbial cells. The important indication is that celluloses do not form humus directly, but indirectly by building the bodies of organisms.

There was also a fine display of soil nemas, showing the ecological types, vertical distribution in the soil, charts of their beneficial and detrimental activities, vectors, etc. A group of microscopes were in place with the nemas arranged for observation.

The whole meeting was splendidly conducted, and will no doubt accomplish great good in all countries. The next international congress of soil science is to be held in Russia, a just recognition of Russia's leadership in the study of the soil. One spirit will be missed at that meeting. GLINKA was not well at the time of the congress, and his recent death leaves a place among the Russian workers that cannot be filled.

THE UNIVERSITY OF CHICAGO, AND SCIENCE SERVICE.



THE MEASUREMENT AND INTERPRETATION OF THE WATER-SUPPLYING POWER OF THE SOIL WITH SPECIAL REFERENCE TO LAWN GRASSES AND SOME OTHER PLANTS¹

J. DEAN WILSON

(WITH THREE FIGURES)

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¹ Botanical contribution from the Johns Hopkins University, no. 84.

I. Introduction

The rate of water absorption and that of transpiration are generally the principal dynamic conditions that control the turgor of ordinary plants, and these rates are consequently of primary importance to plant growth and health. Both rates are subject to influences acting from within the plant and both are influenced also from the outside. Furthermore, they are generally interdependent to a great degree. The transpiration rate is, in many cases, largely determined by the absorption rate, for water cannot be transpired unless previously absorbed. Also, the absorption rate is largely determined, under many circumstances, by the transpiration rate. From the outside the transpiration rate is greatly influenced by the evaporation conditions of the aerial environment, and the absorption rate is similarly subject to the outside influences of the water-supplying power of the soil. Thus the two most important environmental influences acting on ordinary plants, as far as their water relations are concerned, are the evaporating power of the air about their leaves and the water-supplying power of the soil about their roots. The evaporating power of the air may be measured from time to time and its fluctuations may be studied by means of suitable forms of atmometers. The spherical porous-porcelain form of atmometer is specially useful for this purpose, and the radiation (sunshine) influence on evaporation may be estimated by means of porous-porcelain atmometers with blackened spheres (10). The recently introduced "soil-point" method furnishes a promising means for measuring and comparing the various magnitudes of the water-supplying power of the soil for different places and for different depths. This method will be described a little farther on.

To understand the water relations of any plant individual or group of plants, such as those in a garden bed or an agricultural field, it is first necessary to secure suitable measurements of these two dynamic environmental conditions. But it is not primarily necessary, unless details are to be specially studied, to secure precise comparative measurements of these conditions when their intensities lie within the range of safety for the plants. For general ecological and agricultural studies it is at first required only that we know whether the intensities of these conditions surpass the limits of their respective safety ranges, and to know when, for what periods in the growing season, and how much they do so. It is of course necessary to know also the values of the critical limits for the particular plants that are being studied, for these may be expected to differ with the kind of plant and with its size, vigor and general health. There appears to be, however, generally no absolute limit to the safety range of either evaporation or the water-supplying power of the soil, considered separately,

for the limit of one of these conditions for any plant is itself partly determined by the concomitant intensity of the other. For example, a very low water-supplying power of the soil about its roots may suffice for healthy growth of a given plant when the evaporation conditions are also at very low intensities, while even a very high water-supplying power may not suffice to maintain turgor and growth in the same plant when evaporation is very intense.

To approach this somewhat complicated set of dynamic relations it will be necessary first to make general and exploratory surveys of the relation of plant health to evaporation and to the water-supplying power of the soil. Later work may deal with greater detail and with greater precision of experimentation, of statement and of reasoning, but the quantitative and dynamic aspect of plant water relations constitutes a field of research so newly opened and at present so little understood that it must first be entered by means of relatively superficial and reconnaissance surveys. Nevertheless, such surveys must be distinctly quantitative in character and they surely need to approach the subject of plant water relations from the point of view of the dynamics of the processes involved.

The investigations reported in this paper are of the reconnaissance type just suggested. They were planned to give orienting information on the general relation of plant health, wilting, withering, and death, to the general evaporation conditions of the aerial environment and to the water-supplying power of the soil for Baltimore lawns and similar areas in grasses. They were especially planned to study the usefulness and promise of the soil-point method itself, as an aid to physiological ecology and to scientific horticulture, agriculture, forestry, and other lines of study dealing with plant growth. The main aim was to study the fluctuations in the water-supplying power of the soil in comparison with corresponding alterations in plant health, as the latter were recorded in accordance with the results of ordinary observations of color and other aspects of leaf condition. It was hoped that a value of the water-supplying power of the soil might be roughly determined for the evaporation and other climatic conditions dealt with and for certain kinds of plants, above which value vigor and health might be maintained but below which drouth injury might be expected to begin soon unless cloudy weather, rain or irrigation intervened. Attention was to be given to the correspondences between different magnitudes of the water-supplying power of the soil and the several concomitant phases of drouth injury, and especially to any differences that might be indicated between the different plant forms studied, with regard to the characteristic critical magnitudes of the index of water-supplying power for the several forms. The critical value of this index corresponding to such a plant

response as the beginning of wilting, bad withering, or vegetative death, is not the same for all kinds of plants and this value seems to constitute a criterion for comparing drouth resistance in different plant forms, in so far as that resistance is related to the soil-moisture supply. Only the studies on conditions in the open are reported in this paper; those on greenhouse conditions are to be considered in another publication.

This work was done at the Laboratory of Plant Physiology of the Johns Hopkins University, under the guidance of Professor BURTON E. LIVINGSTON, director of the laboratory, and with frequent suggestions and advice from him. Professor LIVINGSTON has helped very much in the interpretation of the results and in their organization and presentation as they are set forth here. The writer is indebted to Professor J. S. AMES, of the Johns Hopkins University, who, as president of the Baltimore Country Club, very kindly made the Club grounds available for a series of tests on the golf course. Thanks are especially due to Mr. ROBERT SCOTT, greenskeeper of the Baltimore Country Club, for much helpful information and for many courtesies.

II. Some considerations for an analysis of the relations of plant health and vigor to evaporation and to the water-supplying power of the soil, as these relations are dealt with in the present paper

When a plant of the ordinary type is continuously and adequately supplied with water from the soil about its roots and when the evaporation conditions affecting its foliage are not too severe, then the plant continues healthy and vigorous and completes its seasonal growth, provided, of course, that the other influential conditions (such as those of temperature, light, supply of nutrient salts, etc.) all lie within their respective intensity ranges for health. Assuming the maintained adequacy of the non-water conditions, a deficiency in the value of the ratio of water supply to water loss may at any time in the growing season bring about the retardation or cessation of enlargement, or the wilting or withering of the leaves, root hairs, etc. The effect of such water deficiency depends upon its intensity and that, in turn, depends on the duration and intensity factors of the two prime environmental moisture conditions, evaporation and the water-supplying power of the soil. For any given set of evaporation conditions that does not overtax the capacity of a given plant to absorb and conduct water (internal conditions), turgor and vigor are maintained as long as the water-supplying power of the soil is greater than a certain critical minimum, a minimum corresponding specifically to the plant and its condition and to the concomitant evaporating power of the air. When the water-supplying power of the soil adjacent to the roots becomes lower than this critical value

the plant begins to lose water somewhat more rapidly than water enters its body, and drouth injury soon sets in. If these relations are maintained, and especially if the water-supplying power decreases with the lapse of time (as it commonly does in a drouth period), even though the evaporation conditions may maintain the same characteristics as were originally assumed, the drouth injury becomes rapidly greater and death and the drying-up of leaves and other parts ensues sooner or later. The same progressive response on the part of the plant may of course be brought about when the water-supplying power of the soil remains unchanged with reference to the root surfaces, but when the evaporation conditions become progressively more severe. Finally, as occurs in most drouth periods, these responses of the plant are greatly accelerated when both influences work together toward an increasing general aridity of the environment; that is, when the evaporating power of the air progressively increases while the water-supplying power of the soil progressively decreases.

Drouth injury, however caused, is generally first noticed as a slowing down and cessation of growth, followed progressively, as a drouth period is prolonged, by the beginning of wilting and its advance through various stages that lead at length to withering and death, first of the leaves, root-hairs and other of the more deciduous parts, but finally of the whole plant, or all but certain dormant portions. Supposing that the effective transpiring surface and the effective water-absorbing surface of the plant remain constant or do not alter in their relation to each other, if we consider the evaporation conditions as remaining alike, day after day, as the supposed drouth period continues, while the water-supplying power of the soil in contact with the plant roots is considered as continually decreasing, there should be a definite value of the water-supplying power that should correspond to each phase or stage of the advance of drouth injury; but, beginning with the first onset of this injury, the time required for any stage of wilting or withering to be reached must differ according to the intensity of the evaporation conditions, the kind of plant and its initial physiological condition, and the rate at which the water-supplying power of the soil decreases. This rate of decrease itself depends, however, largely on the evaporation conditions, which determines in general the rate of water loss from the soil, both directly and through absorption and transpiration by the given plant cover. For a given set of initial plant conditions and a given kind of soil, the time elapsing from the beginning of drouth injury (or perhaps from the last preceding rain or irrigation under some circumstances) to the attainment of any given phase of wilting, etc., should be perhaps approximately proportional to the accumulated total of evaporation from a suitable standard atmometer surface. Such a proportion as this can of course be true

only in cases in which the influential non-water conditions do not become limiting and in which the plant considered does not in the meantime pass into another phase of growth, metabolism or drouth resistance essentially different from that prevailing at the onset of drouth injury. The proposition should perhaps apply approximately to plants in their vegetative phase, which is generally maintained throughout the growing season for the plants of a frequently mowed lawn, and within the period of good temperature and light conditions for growth.

One additional feature of the soil-moisture relations of the ordinary plant needs to be mentioned here; namely, the rôle apparently played by the actual process of enlargement in rootlets and root hairs. This is a phase of the water-relations problem that has only recently been considered at all and the time is not yet ripe for an attempt to discuss it thoroughly. Nevertheless, the formation of new absorbing surfaces in the soil appears to be so important, in the light of recent indications, that it cannot be longer omitted from such discussions as this. Its consideration enters prominently into certain features of the theory and operation of the soil-point method for measuring the water-supplying power of the soil.

It appears highly probable that the roots of a vigorous plant are generally in process of actual elongation, forming new rootlets and root hairs continually, at least as long as the tops are enlarging and producing new transpiring surfaces. It may well be, as has been suggested many times, that there is some sort of physiological correlation between top growth and the enlargement or renewal of the water-absorbing surface of the root system. At any rate, it seems to follow from the DIXON theory (3) of the maintenance of plant water relations (involving the frequent or continuous presence of more or less strain in the water mass of the plant, practically co-extensive with the plant body) that prolonged wilting or withering of foliage must generally be accompanied or shortly followed by a corresponding wilting and withering of root hairs and rootlet tips. CALDWELL (2) has noted the withering of root hairs in connection with serious wilting of the tops of culture plants that had come into this condition with relatively low transpiration rates. It seems logical to suppose that, when growth of tops ceases because of drouth (even before serious wilting occurs), the growth rates of the underground parts are simultaneously greatly retarded or checked. On the basis of such a supposition and its corollaries, one of the dynamic characteristics of a vigorous plant should be a more or less rapid and continual enlargement or renewal of its subterranean absorbing surfaces, and such actual root growth may well be essential to adequate water absorption, especially with moderate or high transpiration rates and relatively low water-supplying powers of the soil. Instead of acting as a

system of stationary absorbing surfaces, taking up water from the soil films as it moves to these surfaces, the healthy root system may act somewhat as a moving wiper; rootlets and root hairs may continually extend themselves into soil regions hitherto untouched by them, absorbing some water from these regions and then expanding into other regions. In this connection it may be noted that root hairs are known generally to wither and die after only a comparatively brief existence. There is thus suggested the very important and fundamental question, apparently not yet seriously treated in the literature, whether the water absorbed by root surfaces has mainly migrated to these surfaces from more or less distant regions of the soil, or whether continually renewed contact with the thicker and more mobile soil-moisture films and wedges is made and maintained by the actual advance of the absorbing surfaces (rootlets and root hairs). This is a question that needs serious study; it is not taken up in the studies here reported excepting in an inductive manner with regard to the formulation of working hypotheses.

Connected with the probability that root systems actually and dynamically operate, in vigorous plants, to grow to the soil moisture and to "wipe it up," is a point of soil physics recently emphasized by PULLING (18), VEIHMEYER (22, 23) and others; namely, that the capillary movement of water (soil solution) in well-aerated soils is very slow indeed and probably quite inadequate to account for the usual rates of root absorption. Combining this condition with that presented in the preceding paragraph, it seems likely that the onset of visible wilting in plant foliage may generally be accompanied or preceded by a retardation or cessation of a very essential wiping-up action on the part of the rootlet and root hair surfaces, thus possibly signifying a very marked and perhaps sudden decrease in the water-absorbing power of the root system. If this picture be a true one, the values of the supplying power of the soil with which ecology and agriculture need primarily to deal are the *initial* values of the water-supplying power, representing the power of the soil to deliver water to a recently placed absorbing surface. It may be that most of the water taken from the soil by a root system enters through the newly formed and newly placed surfaces of capillary contact that are continually resulting from the active penetration of rootlets and root hairs into new regions of the soil. Consequently, the water-supplying power that we need primarily to measure, as the main environmental condition directly influencing the rate of water absorption by plants, may be the supplying power encountered during perhaps only the first hour or two after an adequate absorbing surface has been brought into contact with the soil at the depth to be considered. These possibilities and probabilities are of the utmost importance in interpreting the

results of soil-point tests, for these tests generally give supplying-power indices only for the first hour of exposure of the absorber to the soil.

III. The soil-point method for measuring the initial water-supplying power of the soil

Although the water-supplying power of the soil has been considered for a quarter-century or more (9, 11, 18, 19) as a dynamic environmental condition deserving of very serious attention in plant physiology and in the ecological branches of botanical science, no attempts have been made to measure it in field studies until very recently. Such attempts as have been made have followed the work of LIVINGSTON and KOKETSU (13), done in this laboratory in 1920, in which those writers duly emphasized this feature for the first time and introduced the method thus far used in its measurement. That method is now known as the soil-point method. It has been studied and emphasized by MASON (16), by HARDY (4, 5), by THONE (21), by SHAPOVALOV (20), by LIVINGSTON and OHGA (14) and by LIVINGSTON, HEMMI and WILSON (12). The interesting and promising results secured by the authors of the next to the last reference, from the study of a Baltimore lawn during the summer of 1924, formed the starting-point for the investigations of 1925 and 1926 that are reported in the present publication.

The soil-point method here employed differs but little from that described by LIVINGSTON and KOKETSU and is essentially like that followed by LIVINGSTON and OHGA. Small porous-porcelain cones ("soil-points") are used as standard absorbers. The present form of soil point consists of a hollow, cylindrical portion (1.4 cm. in outside diameter and 2.5 cm. high with a wall about 3 mm. thick), open only at the top but continuous below with a conical portion (5 cm. high, with a wall about 3 mm. thick) that terminates in a point at its lower end. These pieces are externally well water-proofed near the point and throughout the cylindrical part and the upper portion of the conical part, leaving an unwater-proofed absorptive band or zone on the external conical surface. This absorptive zone is 2 cm. wide and has a diameter of 2.2 cm. at its upper margin, 1.5 cm. at its lower margin, and therefore represents about 12 sq. cm. of external absorbing surface.

These porcelain pieces are introduced (air-dry) into the soil at the spots where the water-supplying power is to be determined, an opening having been first made by means of a suitable steel dibble with properly shaped conical point. They are pressed firmly into place, with a slight twisting motion, and are left in position generally for one hour. (LIVINGSTON and KOKETSU employed a two-hour period, but the shorter period is more satisfactory.) They are then removed, brushed quickly to remove adhering soil

particles, and returned to a corked glass container that will accommodate either one or two instruments. The original weight of each container with its one or two instruments is known at the start and the difference between that and the corresponding weight after exposure represents the weight of water gained during the exposure. The amount thus gained for each instrument, recorded in milligrams in these studies, is considered as a measure of the initial water-supplying power of the soil at the point where the test was made. The solute content of the water absorbed is ignored as insignificant. By introducing the instruments vertically into the soil from the otherwise undisturbed surface of the ground, the middle of the absorbing zone may be brought to lie about six centimeters below the ground level. For tests at greater depths a preliminary excavation is made.

In order that the amount of water absorbed in an exposure period may represent the power of the soil to supply water during that period, it is of course necessary that the instrument shall possess an absorbing power for water greater than the supplying power to be measured and that this relation shall be maintained throughout the period of exposure. The instrument must take up water as fast as the liquid comes to the absorbing surface. It is patent that the absorbing power of porous-porcelain decreases rapidly as water is absorbed and the results secured by the soil-point method from very moist soils are therefore surely always relatively much too low, as has been emphasized by LIVINGSTON and KOKETSU. This consideration has formed a part of another study by the writer, not here reported on, and it seems to be clear that the special relation just stated is maintained for at least an hour when these instruments are placed in soils that are not obviously rather moist. The readings of the present study are probably satisfactory indications of the water-supplying power of the soil in all cases where their values are less than about 600 mg. For higher values an increment of correction should be added, its magnitude being of course progressively larger as the observed value is higher.

In these studies no attempt was made to correct the higher values, but such corrections may perhaps be introduced at a later time, when the results of further studies on the soil-point method may be brought together. For present purposes, and probably for nearly all purposes of ecological and physiological inquiry for a long time to come, such corrections are unnecessary, for the reason that soil water-supplying powers that are above the value where a correction of this sort begins to be requisite appear generally to be adequate to support vigorous growth in upland plants of temperate regions. In interpreting the higher values, for wet soils, it is mainly desirable only to note that the value is well above the danger-point for the plants considered, but a series of these higher values secured at successive

times does clearly indicate the rapid falling-off in the water-supplying power of the soil after the occurrence of a rain or other wetting of the soil. For lower values the results may apparently be considered as approximately comparable, and the instrument is therefore adequate for comparing the critical water-supplying-power values that correspond to the beginning of wilting, etc., for different plant forms. As will be seen in the following accounts, the plants studied generally grew vigorously as long as the supplying-power index was above about 500 and special interest in the values of this index as related to drouth effect is mainly confined to a range below 500. Further details in this general connection are to be gathered from succeeding pages, where actual readings are under consideration.

Since the amount of water absorbed by a soil point is influenced by the area of the absorbing zone of the instrument, as well as by the duration of its exposure to the soil and the water-supplying power of the latter, the actual values of soil-point readings need always to be considered with reference to the particular form of soil point used. (The instruments employed by LIVINGSTON and KOKETSU were slightly different from those now in general use, and they employed a two-hour period instead of the one-hour period used in the present studies. MASON and HARDY used ordinary, mechanically sharpened lead-pencils as soil points.) It should be possible to bring into a homogeneous series readings made with different absorbing surfaces, by dividing each reading by the area of the particular surface employed in securing it, thus expressing the water-supplying power of the soil always with reference to the same extent of area of absorption. This sort of treatment will soon be desirable but it is not generally resorted to in the present report, since the data here considered are all derived from the same type of soil point, with only very slight variation in the area of the absorbing zone. Since this area uniformly is about 12 sq. cm. in extent, the water-supplying power for a square centimeter of cross-sectional area of the soil may be secured in any case by dividing the given reading index value by 12.

The soil-point method introduces a modification in the packing of the soil at the very place where readings are to be taken. The insertion of the steel dibble prepares the opening and the soil surface for the application of the soil point itself, and this operation is performed by compacting to a considerable extent the soil adjacent to the dibble. This process of compacting must generally result in decreasing the volume occupied by unit volume of undisturbed soil, and consequently in generally making the volumetric water content somewhat larger after compacting. In general, this modification increases the initial water-supplying power of the soil, and soil-point readings are therefore generally somewhat higher than they would have been had the soil against the absorbing surface not been artificially

compacted. While this feature of the soil-point method will require attention as the method comes into more general use, the "error" is presumably approximately uniform, and the empirical outcome of such studies as those reported in this paper shows that it may safely be ignored for most field work. It amounts to this, that any soil-point determination indicates an initial water-supplying power for an aerated soil somewhat higher than the actual supplying power at the given place before the soil was compacted by the dibble.

As has been said, the soil-point readings given in the following pages are expressed as milligrams of water delivered to the absorbing surface of a single instrument in one hour of exposure, and they generally refer to the six-centimeter depth. Considered as numerical indices of the water-supplying power of the soil, they are regarded simply as relative values, the actual unit of weight being generally omitted. Thus, for a given reading of 120 mg., for example, the corresponding index value is simply 120.

IV. A seasonal study of Kentucky blue-grass and white clover

METHODS AND OBSERVATIONS

It is generally appreciated that the condition of a plant at any time has come about under the influence of more or less fluctuating environmental conditions that have been acting since the plant began its growth. This plant condition may be regarded as the summed or integrated effect of all these past influences, harmful as well as beneficial, but the effect of the more recent environmental conditions is more apparent and more easily studied than are the effects of less recent influences. Among the environmental conditions that produce quick and conspicuous responses in plants during the growing season are especially those of soil moisture and evaporation. The studies here reported deal with the wilting and drying of plant leaves resulting from drouth periods in the growing season, and with the recovery of the plants when moisture conditions become again favorable for growth.

A well-established lawn, of grasses and other plants that grow well in the region considered, should offer an excellent opportunity for studying these water relations. Lawn grasses are generally shallow-rooted, being consequently especially sensitive to changes in soil moisture and evaporation, for they are dependent for their water on the surface layer of the soil, which is subject to great and rapid variations in water content as the weather changes. When, in the growing season, the supply of soil moisture becomes insufficient to maintain turgidity in the upper parts the leaves wilt and, if this insufficiency persists long enough, death and drying-up ensue. Although dead grass leaves generally remain in position, they draw prac-

tically no water from the roots. The subterranean buds and many roots may remain dormant for a time, and new leaves are quickly produced when the moisture supply becomes once more adequate for growth.

Lawn grasses are specially suitable for observation in regard to moisture conditions, on account of the fact that, as drouth proceeds, the leaves change color from green through several shades to brown, so that the whole lawn conspicuously and progressively alters its color. Also, when the supply of soil moisture becomes again adequate for renewed growth the whole lawn promptly becomes green, through the production of new leaves. Several other lawn plants, besides the grasses, although their changes may not be so readily observed, show somewhat parallel responses to alterations in the moisture conditions of the soil and air.

The lawn referred to in this part of the present study is in the central quadrangle of the Johns Hopkins University grounds at Homewood, Baltimore. The lawn plants were strong and vigorous in the spring of 1925, when the observations were begun, the lawn having been started nine years earlier. Weeds were very infrequent except for several small areas of crab-grass (*Syntherisma sanguinalis* L.), which became conspicuous in July and disappeared in the early fall. Kentucky blue-grass (*Poa pratensis* L.) is the main plant throughout most of the lawn, although some areas are densely populated by white clover (*Trifolium repens* L.), which appears in the spring somewhat later than the blue-grass and disappears earlier in the fall.

The soil of this lawn is a rather heavy loam (Sassafras loam) with only a little organic matter. The area is level but well drained on account of its elevated position, and it is freely exposed to wind and sunlight. Even with heavy rains there is but little surface run-off. The water-holding capacity of the surface soil, determined by the HILGARD method, is from 48 to 54 per cent. of its volume (51 to 56 per cent. on the dry-weight basis).

The series of observations here considered was begun April 1, 1925, and continued until the following October 31, thus covering a period of 214 days. This period was a little longer than the actual frostless season for 1925. The last spring frost occurred on April 7, and the first autumn frost occurred October 11, but the second autumn frost was delayed until October 28. The latter part of March was favorable for the initiation of plant activity, and the lawns remained green until the middle of December. Since the plants studied are not specially sensitive to frost, the period of these observations may closely represent the growing season for the year considered. The average or normal frostless season for Baltimore extends from April 4 to November 3, a period of 213 days (15) and the period of the present observation is seen to be almost the same as the normal.

The water-supplying power of the soil was determined by the soil-point method, at intervals of from two to six days, depending upon the condition

of the plants and the weather. At each observation four soil points were used, these being placed, with the aid of a steel dibble, at spots selected to be representative of the general condition of the lawn. Care was taken to avoid by a distance of at least 30 cm. every one of the little pits remaining from earlier tests. Each instrument was always so placed that the middle of the unwater-proofed portion was about 6 cm. below the surface of the soil. The time period for a test was an hour in every case.

The observation period was not shortened when the soil was very wet, as was done by LIVINGSTON and KOKETSU. It therefore follows, as has been mentioned, that the readings secured when the soil was wet are more or less too low, if direct comparison is made between them and readings from drier soils. Since, however, such direct comparisons are not needed in the present study of lawn conditions, no attempt has been made to care for this point. The critical soil-moisture condition in which we are specially interested (that is, the point at which the plants begin to show drouth injury) corresponds to a very low soil-point reading. When the reading is above 500 mg. it is safe to consider that the plants are not suffering from soil-moisture deficiency, and any higher reading is to be taken as an indication of adequate water supply. In the present connection it is not necessary, excepting in a very superficial way, to make quantitative comparisons between different readings when all are above this approximate limit. Our main interest is in index values below 500.

Upon removal from the soil at the end of the one-hour period each soil point was quickly brushed to remove adhering soil particles and was then immediately returned to the weighing tube. Two of the cones were usually weighed together in the same tube, both before and after use. Record was finally made of the average gain per instrument, expressed in milligrams. But the numerical values are frequently considered simply as relative indices and are consequently stated as abstract numbers in the following pages, as has been said. Each value is the average of four tests.

At each determination of the water-supplying power of the soil notes were made on the hardness of the soil, the general appearance of the lawn plants, and occasionally on the general condition of the surrounding vegetation. On some small areas of the lawn where the effects of drouth were specially pronounced the successive appearance and disappearance of certain species during the season were particularly studied. Attention was also given to changes in the make-up of the visible plant population as the season advanced, especially with reference to blue-grass and white clover.

The growth condition of the lawn plants, as judged by appearance, was recorded at the time of each observation. Five different conditions were arbitrarily chosen for use in making these records. They may be defined as follows, the numbers representing score values:

4. Plants in excellent condition.
3. Plants in good condition.
2. Leaves wilting and changing color.
1. Leaves very brown.
0. Leaves dead.

"Excellent condition" denotes maximum greenness and luxuriant, thick growth. "Good condition" denotes less vigorous plants, but this condition is generally regarded as satisfactory for lawns. "Wilting and changing color" denotes the wilting of many leaves and the conspicuous loss of greenness by some. "Very brown" denotes the condition when about half of the leaves are brown. "Dead" denotes the almost entire lack of any green leaves, the lawn appearing wholly brown or tawny.

Since other environmental conditions are influential in determining the critical values of the soil-moisture conditions, with reference to wilting and to the general health of plants, evaporation and sunshine records were kept throughout the season. LIVINGSTON standardized radio-atmometers (10) of the spherical type were used for securing evaporation and sunshine data. The instrument consists of two porous-porcelain spheres, one black and the other white, operated side by side. The corrected water loss from the white sphere for a given period is taken as a measure of the average evaporation intensity for that period and exposure, aside from the influence of sunshine. The corresponding corrected loss from the black sphere is taken to indicate the total evaporation intensity, including the effect of sunshine. A value for the effect of sunshine, as it influences evaporation from the standard spherical surface, is secured by subtracting the corrected loss by the white sphere from the corresponding corrected loss by the black sphere. Three of these double instruments were operated simultaneously and read daily at six o'clock in the evening, the average corrected loss from the white spheres (e), the average corrected loss from the black spheres (E), and the average difference ($E-e$) being recorded for each day. The instruments were freely exposed, 1.5 m. above the ground and 35 m. from the edge of the lawn area studied.

Data on daily sunshine duration, air-temperature, and precipitation were secured from the Baltimore office of the United States Weather Bureau, which is situated about 4 miles south of the Homewood grounds. In spite of this difference in location the Weather Bureau data are surely valuable in connection with this study.

All of the numerical data for this seasonal study are presented in table I. Column one shows the dates of observation and column 2 shows the length of each period between soil observations. In columns 3, 4, and 5 are given the atmometer averages as above defined, the number given in each case

TABLE I

EVAPORATION, SUNSHINE, TEMPERATURE AND PRECIPITATION VALUES, TOGETHER WITH VALUES
OF THE WATER-SUPPLYING POWER OF THE SOIL AND INDICES OF PLANT
CONDITION FOR THE HOMEWOOD LAWN, SUMMER OF 1925

FOR EXPLANATION SEE TEXT

DATE	EVAPORATION VALUES, AVERAGES PER DAY				TION PER DAY AVERAGE SUN- SHINE DURA- (L)	MEAN DAILY AVERAGE TEM- PERATURE (T)	TOTAL PRECIP- ITATION (P)	SOIL-POINT READING	CONDITION OF PLANTS	
	NO. OF DAYS IN PERIOD	BLACK SPHERE (E)	WHITE SPHERE (e)	E-e					BLUE- GRASS	WHITE CLOVER
April		cc.	cc.	cc.	hr.	Deg. F.	in.			mg.
1	—	23	19	4	2.0	47	0.01	1	0	1667
2	—	—	—	—	—	—	0.04	—	—	—
4	3	27	18	9	7.3	50	—	2	0	1915
7	3	44	32	12	13.0	47	—	3	0	515
10	3	41	34	7	8.0	56	0.15	3	1	315
13	3	50	39	11	13.0	60	—	3	2	185
14	—	—	—	—	—	—	0.61	—	—	—
15	—	—	—	—	—	—	0.03	—	—	—
16	3	41	31	10	9.3	60	—	3	3	196
17	—	—	—	—	—	—	0.34	—	—	—
19	3	25	18	7	6.3	58	0.18	4	4	655
22	3	34	26	8	10.7	50	—	4	4	156
25	3	34	25	9	8.7	68	0.45	4	4	460
28	3	30	23	7	10.3	67	0.47	4	4	2036
29	—	—	—	—	—	—	0.01	—	—	—
30	—	—	—	—	—	—	0.37	—	—	—
May										
1	3	22	17	5	5.3	49	—	4	4	1705
3	—	—	—	—	—	—	0.01	—	—	—
4	3	39	30	9	9.3	57	0.08	4	4	702
7	3	27	20	7	8.3	53	—	3	4	333
10	3	29	20	9	8.3	59	—	3	4	139
11	—	—	—	—	—	—	0.43	—	—	—
13	3	18	14	4	5.0	61	—	3	4	448
16	3	37	28	9	11.0	63	—	3	4	216
19	3	40	30	10	10.3	62	—	3	4	88
22	3	52	41	11	13.0	67	—	2	3	45
24	2	62	53	9	10.5	74	1.17	1	2	24
25	—	—	—	—	—	—	0.01	—	—	—
26	2	22	14	8	7.5	52	—	2	3	1015
29	3	41	29	12	10.7	62	0.16	3	4	380
June										
1	3	48	34	14	12.3	72	—	2	3	93
3	2	54	40	14	13.0	84	—	1	2	52
5	2	62	48	14	13.5	88	—	0	1	32
7	2	62	48	14	11.5	88	—	0	1	12
9	2	38	27	11	11.5	81	0.04	0	0	7
11	2	67	53	14	13.0	73	—	0	0	9
13	2	55	41	14	12.0	71	—	0	0	7
15	2	53	41	12	11.5	82	—	0	0	8
18	3	54	43	11	10.0	78	—	0	0	8
22	—	—	—	—	—	—	0.06	—	—	—
23	5	51	41	10	10.6	78	0.12	0	1	7
24	—	—	—	—	—	—	0.11	—	—	—
25	—	—	—	—	—	—	0.54	—	—	—
26	3	38	27	11	8.0	75	—	1	1	93
29	3	41	32	9	6.0	77	0.11	1	1	52
30	—	—	—	—	—	—	0.03	—	—	—

DATE	NO. OF DAYS IN PERIOD	<i>E</i>	<i>e</i>	<i>E-e</i>	<i>L</i>	<i>T</i>	<i>P</i>	BLUE- GRASS	WHITE CLOVER	SOIL-POINT READING
July		cc.	cc.	cc.	hr.	Deg. F.	in.			mg.
3	4	51	37	14	12.0	74	1	1	16
4	0.21
5	0.03
7	1.24
8	5	51	36	15	11.0	82	1	1	264
10	0.15
11	0.11
12	4	33	22	11	5.8	82	0.08	1	1	20
14	2	59	44	15	8.5	77	1	1	15
15	0.02
16	2	37	25	12	6.0	79	0.18	1	2	227
20	4	45	31	14	10.8	74	1	2	55
21	0.10
22	0.61
24	4	34	24	10	8.0	76	2	3	1846
25	0.56
26	0.79
27	3	27	16	11	8.3	78	0.06	3	3	1709
28	0.18
31	1.25
Aug.										
1	5	39	26	13	10.2	72	4	3	1752
5	0.47
6	5	21	12	9	7.6	73	4	4	707
8	0.01
9	0.04
10	4	33	21	12	11.5	79	0.26	4	4	348
12	0.04
14	4	24	17	7	7.3	78	3	4	138
18	4	45	32	13	13.5	77	2	3	82
20	0.09
21	0.73
22	4	30	20	10	9.0	74	3	3	200
26	4	39	25	14	13.3	72	3	3	45
29	3	48	34	14	12.7	68	1	2	35
31	0.77
Sept.										
1	3	40	29	11	11.3	77	1	3	383
4	3	37	27	10	9.3	77	2	3	142
6	0.21
10	6	33	23	10	8.2	77	1	3	81
12	0.19
13	0.08
15	5	33	23	10	8.2	79	0.08	1	3	117
18	3	29	19	10	9.3	75	1	3	104
21	0.01
22	4	41	31	10	8.5	74	1	3	61
26	4	29	21	8	8.0	61	0	2	35
28	0.18

TABLE I (Concluded)

DATE	NO. OF DAYS IN PERIOD	E	e	$E-e$	L	T	P	BLUE- GRASS	WHITE CLOVER	SOIL-POINT READING
						Deg. F.				
Oct.		cc.	cc.	cc.	hr.		in.			mg.
1	5	29	20	9	4.4	69	0	2	39
2	0.77
3	0.08
4	0.92
5	4	28	21	7	4.3	63	1	2	264
9	4	21	11	10	6.8	56	0.22	2	3	826
12	3	22	17	5	6.3	47	0.05	3	3	596
13	0.04
14	0.57
16	4	18	14	4	4.3	60	0.30	4	3	621
17	0.02
21	5	24	18	6	5.8	53	4	3	708
22	0.33
24	1.14
25	0.56
26	5	13	9	4	3.8	50	4	3	834
30	0.43
31	5	25	20	5	7.6	41	4	3	1240
Nov.										
10	3	2	1618
19	3	2	2010

being the *mean* of the daily averages for the corresponding period between the dates of the soil observations; these means are expressed as cubic centimeters of water loss per sphere per day. In column 6 are given the average mean daily values of sunshine duration (L) for the same observation periods, the duration being measured in hours. Air-temperature data (T , degrees F.) are presented in column 7 in the same way as are the evaporation values already noted, each value being the average of the daily means for the corresponding period. In column 8 is given the *actual* rainfall value (P) for each day when precipitation of 0.01 inch or more occurred. The values of the water-supplying power of the soil (S , expressed as milligrams of water absorbed per soil point in the one-hour exposure) are given in the last column of the table. In columns 9 and 10 are indicated, by the numerical symbols or scores described above, the growth-condition of the two main plants, Kentucky blue-grass and white clover, respectively, at the several times of observation.

The growing season of 1925 was marked by unusual weather for Baltimore. The spring was somewhat drier than usual. June established a record for high air temperature, the average daily mean for the month being

2° F. above the highest previous record for this station. On July 1 the accumulated rainfall deficiency since January 1 was 7.4 inches. June was also a month of very high evaporation, the highest of the twenty-one months of the three frostless seasons, 1923, 1924, and 1925, for which records are available. As a result of the long period of drouth and hot weather the vegetation of the Baltimore region was showing marked evidence of drouth injury in mid-July; most unirrigated lawns showed practically no green color. Many of the other plants of the Homewood grounds had lost much of their foliage or had even succumbed to the drouth in some cases. For example, many of the hemlocks in the hedges of the Botanical Garden, which are regularly pruned to a height of about 80 cm., were actually killed and had to be replaced subsequently. The vegetation generally returned to its normal condition by the end of July. Early in August a second but less pronounced drouth set in and continued with increasing severity until the end of September, at which time the accumulated deficiency of rainfall since the beginning of the year amounted to 11.5 inches. In spite of several rains in August and September the vegetation, and especially the lawn plants, showed increasing drouth effect, and the lawns appeared brown throughout the latter month. The September drouth was accompanied by unusually high evaporation for the month as well as by great deficiency in precipitation. October began with several days of rain, with the occurrence of which the second drouth period promptly came to an end. A high water-supplying power of the soil was evident by the end of the first week of October and the lawn plants rapidly came into good condition, which was maintained until browning due to low temperatures finally set in about December 15.

DISCUSSION OF RESULTS

Some of the data of table I are presented in the graphs of figure 1. Dates from April 1 to October 31 are indicated by equal spacing on the base line, and the various values that are to be compared are shown as ordinates. The data for November 10 and 19 are not shown on the graphs. The ordinate scale for any single graph is of course uniform throughout, but the scales for the different graphs are not generally quantitatively related, being arbitrarily chosen, merely for convenience in bringing out the successive rises and falls of the several values plotted.

The continuous broad line represents the soil-point readings (S), taken from the last column of table I. In this graph, as well as in the graphs for evaporation and plant condition, the tops of the adjacent ordinates are arbitrarily joined by straight lines for ease in reading. In the case of the precipitation data (table I, column 8) the lengths of the vertical lines represent the respective ordinates and no connecting lines are introduced. The posi-

tions and lengths of these lines consequently present the dates and amounts of rain. The dotted line presents the data for total evaporation (E , column 3, table I). The growth condition for blue-grass (column 9, table I) is shown by the continuous narrow line and that of white clover (column 10, table I) by the broken narrow line. In these two graphs the condition indi-

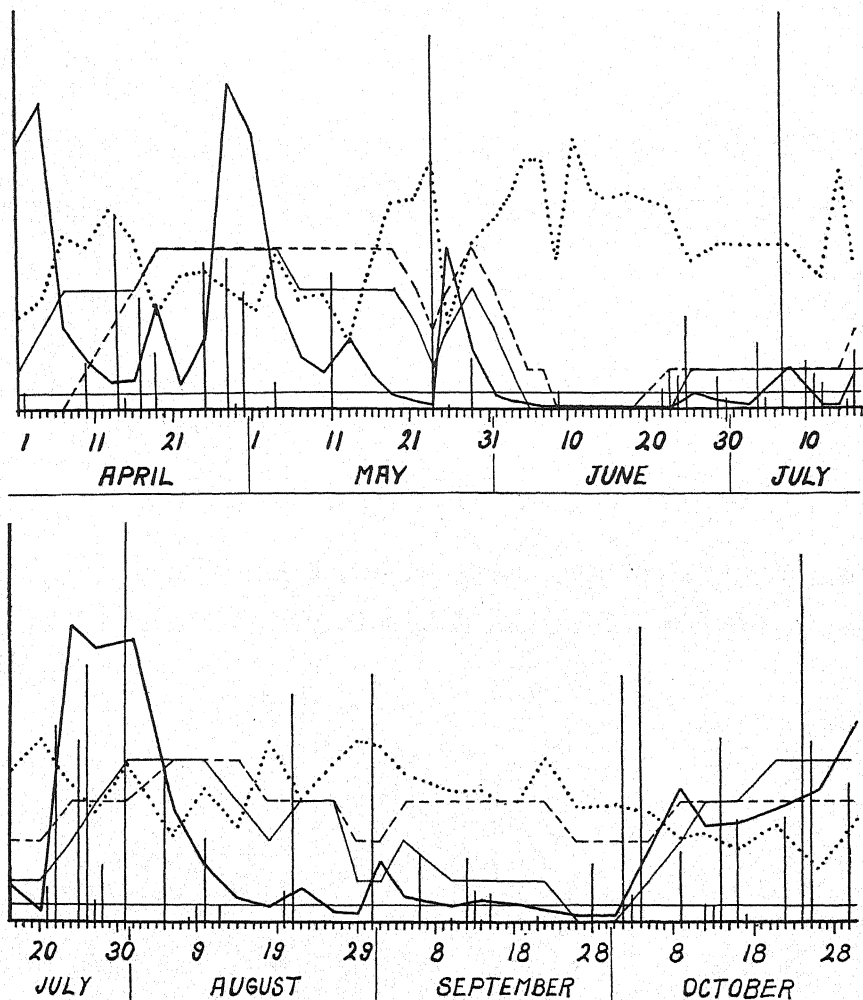


FIG. 1. Graphs of data given in table I, showing fluctuations, throughout the season, in water-supplying power (broad continuous line), in evaporation (dotted line) and in condition of Kentucky blue-grass (narrow continuous line) and of white clover (narrow broken line). Precipitation is shown by positions and lengths of vertical lines. Part II of this figure continues Part I.

cated by 0 is plotted on the base line and conditions 1, 2, 3, and 4 are respectively indicated at four equally spaced intervals above. Some of the prominent features brought out by these graphs will now be mentioned.

The water-supplying power of the soil was indicated as high (above 1000) at the beginning of the period but it soon fell rapidly to low values (between 200 and 150), increasing again to the high values recorded for April 28 and May 1 (above 1700). Another rapid fall then occurred and the value for May 24 is only 24. The heavy rain of May 24-25 caused another rise in the water-supplying power but this was only temporary and the first prolonged drouth period of the season had set in by June 1, with a value of 93. With minor fluctuations and in spite of the heavy rain of July 7, the observed value remained low until July 22, the first drouth period coming to an end with the rain of that date.

Turning to the graphs for plant condition it is seen that both blue-grass and white clover were in poor condition at the beginning of the season, because spring growth had not yet fully set in at that time; although the soil-moisture conditions had been excellent, yet the preceding low temperatures had retarded development. Both plants were in excellent condition on April 19 and their condition continued good until about May 24, on which date was clearly indicated the beginning of drouth retardation. This effect was immediately brought to an end, however, by the rain of May 24-25, to which both plants responded, as is indicated by the observation of May 29. Both plants showed evidence of marked drouth effect during the first week of June and their leaves had all died by June 9. They recovered slightly with the rains of June 22-25, but showed no considerable growth until the end of the first drouth period, after July 22.

After the temperature conditions became favorable for growth in the spring the plant condition, as here indicated, followed the water-supplying power of the soil, but with a considerable lag in time. It is interesting to note that the period from June 9 to June 22 is characterized by exceedingly low values of the soil-moisture index as well as by very poor condition of both plants. It is also to be noted that small fluctuations occurred in the soil-moisture index without being accompanied by visible plant responses.

The rainy period that followed the first drouth quickly brought both plants into good condition. Both again showed drouth effects on August 29 but they quickly showed some recovery after the rain of August 31. In the meantime the water-supplying power of the soil, which was very high at the end of July, fell to the low values indicated for the second drouth period, which in turn ended with the rain of October 2. A temporary increase in the soil-moisture value is indicated for September 1, but this second drouth period may be considered as practically continuous until October 2. With the exception just mentioned, the values of the soil-point readings

throughout this drouth period were generally about 100 or below. During this period the blue-grass plants were affected increasingly and appeared dead during the last 6 days. While the clover plants showed considerable injury at the end of this drouth, their foliage was not completely killed. This difference between the two plants may be due to greater drouth resistance on the part of the clover; it is probably related to the deeper-rooting habit of this plant.

After October 2 the soil-moisture index rapidly increased and for the rest of the season remained above 296 (the value for October 12). The blue-grass regenerated and was in excellent condition from October 21 on; but the clover did not reach the condition designated as excellent. Perhaps it was more seriously injured by the preceding drouth than was the blue-grass, even though it did not show it at the time of that drouth, or it may be that this failure of the clover to recover in the autumn was due to temperatures inadequate for good growth of this plant. General observation supports the idea that clover requires higher temperatures than does blue-grass.

Considering in particular the periods of high water-supplying power, it is of course obvious that each of these periods begins with a heavy rain or with the accumulated effect of several lighter rains. The effect of a rain in increasing the soil-moisture content, and consequently the water-supplying power of the soil, is dependent not only upon the amount of precipitation but also upon the kind of soil, its physical condition and its previous water content, the vegetation cover, and the rapidity of the precipitation as related to the slope of the soil surface. For all cases where the influence of rain is shown by the data of the present study the kind of soil and its physical condition, as well as the vegetation cover, were generally about the same. As to the rate of rainfall and the slope, it is clear that some of the water falling upon any small area of soil runs off to adjacent lower areas, if such are available, whenever precipitation is more rapid than absorption of water into the soil where the rain falls. It commonly occurs, even on lawns that are approximately level, as this one is, that the soil in slight hollows receives considerably more water from a shower than does the rest of the area. This was frequently noted on the lawn here studied. It was also noticed that the slight hollows were generally apt to be dominated by clover, although not to the entire exclusion of blue-grass. With the exception of these slight hollows, blue-grass is generally dominant throughout this lawn.

A very slow rain may be entirely absorbed where the water falls, in which case these phenomena of surface drainage would not occur. For any given kind of soil, physical condition, vegetation cover and slope, there should be some maximum rate below which precipitation water absorbed by the soil

at any point should be equivalent to the actual precipitation at that point. Since the vegetation cover usually retards superficial flow of accumulated, unabsorbed water, it appears that differences due to this kind of drainage should be less pronounced where vegetation is dense than where it is sparse.

With these ideas in mind it is interesting to examine the supplying-power graph of figure 1, in comparison with the rainfall data. It may be noted in many cases that rapid heavy rains had comparatively little effect in altering the supplying-power value, while in other cases rains of less total depth but more gradual, or several relatively light showers occurring at short intervals, increased the supplying-power value at the 6-cm. depth to a much greater extent. Several light rains of late April resulted in very great increase in the water-supplying power of the soil. The rapid, heavy rain of May 24 increased the supplying-power reading from 24 (May 24) to 1015 (May 26). On July 7 a short, rapid shower of about the same depth had but little effect, increasing the water-supplying power only from 16 (July 3) to 264 (July 8). The light shower of July 21, followed the next day by a rain of medium depth, raised the soil-moisture value greatly, from 55 (July 20) to 1846 (July 24). Rains totalling almost 3 inches in the next ten days maintained the water-supplying power at a high value. The rains of August 20 and 21, totaling 0.82 in., resulted in only a slight increase in the supplying-power reading, from 82 (August 18) to 200 (August 22). Similarly the rain of August 31 resulted in raising the water-supplying power only from 35 (August 29) to 383 (September 1). The relatively slight increase in the water-supplying power from 32 (October 1) to 264 (October 5) was the result of rains occurring on October 2, 3, and 4, totalling 1.77 in., but the slight rain of October 9 raised the water-supplying power to 826. The last two instances furnish an example of a marked influence exerted by the water content of a soil before the occurrence of a given rain in determining the influence of that rain on the water-supplying power at the 6-cm. depth, as observed shortly after the rain has ceased.

It is interesting to note that the four maxima of water-supplying power (the last one not shown on the graph) all have nearly the same value (1915, 2036, 1846, and 2021). No readings of the supplying power were made immediately after a rain, several hours being allowed to elapse in every case. The similarity of these values is apparently due to the fact that the surface layer of the lawn soil, when these maximum readings were secured, had a critical water content determined by the nature and condition of this layer and by the deeper layers. Evidently this soil at the 6-cm. depth always comes to this critical water content and to the corresponding water-supplying power within a few hours after each saturating rain, as soon as capillary drainage has ceased. The critical water content here indicated is

familiar to soil scientists and has been called the maximum field capacity for the depth and location considered.

Following each period of high water-supplying power of the soil this power is seen to decrease and it is interesting to study the data and graph for the periods of decrease. Between April 4 and April 13 the supplying power fell from a maximum to 185 and the slight rain on the tenth of the month does not appear to have altered the rate of decrease in the value in question. It is seen that this descending portion of the graph is steep at first and then flattens in its lower portion. As would be expected, the slope of this part of the graph, especially in its upper region, shows some relation to evaporation. During this period of nine days the supplying power was decreased by 1730 and in the same period the evaporation amounted to 402 cc., or the supplying power decreased at the average rate of 4.3 for each cubic centimeter of evaporation from the black atmometer sphere. Between April 28 and May 10 the water-supplying power was decreased by 1899 and the form of the graph is here closely similar to that for the case just considered. The total evaporation for this twelve-day period was 354 cc. and the average rate of supplying-power decrease is 5.3 per cubic centimeter of evaporation. The rain of April 30 somewhat retarded the decrease. The graph for the fall in supplying power that follows the low maximum of May 26 is seen to have approximately the same form as that which characterizes the lower portions of the two downward slopes already considered. The downward slope of the supplying-power graph for the period from August 1 to 14 (13 days) exhibits the same general form as the first two of these downward slopes. The decrease in this case was 1594 and the corresponding total evaporation was 378 cc., or the average decrease was 4.2 per cubic centimeter of evaporation. The rains of August 5 and 10 were without marked effect in altering the form of this slope.

Summarizing the last paragraph, it may be said that, for the lawn studied, the decrease in water-supplying power following a maximum forms a graph which has generally about the same slope in all cases, the average rate of fall from high values to rather low ones being about 4.6 per cubic centimeter of evaporation (from the black atmometer sphere) for the period of decrease. Data from three similar cases in greenhouse cultures of grass, with the same kind of soil and with atmometers exposed close to the soil surface, have given much the same slope, with an average decrease in the water-supplying power of 5.5 for each cubic centimeter of total evaporation. It should be noted that some rain occurred in every case during the period of the downward slope in the open and that there was no irrigation in the greenhouse. The effect of rain in one of these drying-out periods would of course be to make the slope of the graph less pronounced, giving the ratio

here considered a lower value than it would have in the absence of rain effect.

It is suggested that the ratio value secured by dividing (*a*) the water-supplying-power decrease (from the value corresponding to the maximum field capacity to a low value near the beginning of drouth effect) by (*b*) the total water loss from a standard atmometer for the given exposure and for the same period, may be a valuable ecological index. Such a value must include influences of both the soil and the climate and may prove useful in the analysis of the general relations that obtain between plants and their environmental conditions.

From observations made in the summer of 1924, on a lawn area very similar to the one here studied and with soil points of the same type as were here used, LIVINGSTON and OHGA (14) came to the tentative conclusion that a supplying-power value of 100 (0.10 g. on their scale) is critical for lawn grasses in this region. They say "... that the physiologically critical value of the water-supplying power of the soil, as measured by these soil points and as indicated by our grasses, ... appears to have been about 0.10 g. ... and that about four or five days with soil-point values below 0.10 g. should be expected to bring about marked discoloration ...". On the basis of many observations made in the present studies, this value of 100 appears to be a close approximation to the critical point in question, below which lawn plants, at least blue-grass and white clover, would begin to suffer within from 3 to 5 days if no rain occurred and with evaporation rates of about 50 cc. per day from the standard black sphere, which is the usual summer rate for Baltimore.

It should of course be understood that the critical value indicates that the soil in question can supply 100 milligrams of water in one hour to the absorbing surface of the porous-porcelain cone used. Since this absorbing surface is of approximately 12 sq. cm., it is possible to make an approximate estimate of the critical water-supplying power in terms of any standard area of absorbing surface. For example, a supplying power with an index value of 100, as here expressed, may be taken as approximately equivalent to a supplying power of 8.3 mg. of water per *square centimeter*, 833 mg. per *square decimeter*, or 83 g. per *square meter* for the first hour.

This critical value of the water-supplying power of the soil, as empirically indicated by the results of LIVINGSTON and OHGA and by those of the present studies, may possibly bear some definite relation to the critical value corresponding to the onset of wilting, etc., or the "wilting point" of BRIGGS and SHANTZ (1), but the two are not at all the same. These lawn plants were apparently not generally actually injured by the occurrence of water-supplying powers of 100 at the 6-cm. depth. Injury was gen-

erally manifest, however, within a very few days after this occurrence, providing no rain fell in the meantime. Many of the absorbing roots were doubtless much deeper than this depth of testing and the critical value for the shallow depth is to be regarded simply as a general indication of the conditions that actually obtained about the roots when injury was imminent. Furthermore, it must be repeatedly emphasized that the critical value here considered, as well as that for any given stage of wilting or subsequent injury, must depend in general not only on the kind of plant dealt with and its condition, but also upon the intensity of evaporation.

An examination of figure 1 will be interesting in connection with the critical value 100, which is represented in the figure by a horizontal line just above the base and extending throughout the whole period. We are specially interested in noting the times or points at which the water-supplying power of the soil fell below the critical value. This occurred for the first time on May 19 and blue-grass was observed to be affected 3 days later, while white clover showed marked signs of drouth effect after an additional two days. Evaporation was unusually high for May 19 to 24. The very heavy rain of May 24 replenished the soil moisture and the condition of the plants was temporarily improved. On June 1 the water-supplying power was again observed to be below the critical value, when blue-grass was wilting and clover was beginning to show some effect of drouth. Two days later clover was unquestionably injured and blue-grass was very brown. After the heavy rain of July 7 the soil-moisture index was considerably above the critical value on July 8, but was again below it on July 12 and continued so until the rain of July 16. The condition of the plants, which was very poor at the beginning of this four-day period, showed no change. The index value again fell below 100 on July 20 and in this period neither plant showed any change according to the numerical scores representing plant condition, although clover was noted to have been slightly improved by the rain of July 16. The next occurrence of a water-supplying-power index below 100 was on August 18. On that day blue-grass was wilting and clover showed some drouth effect. The supplying power was well above the critical value on August 22 but again fell below it on August 26, and both plants showed marked drouth effects by the 29th. After a temporary increase, the supplying-power value fell again below 100 on September 10. During the preceding 6 days the condition of blue-grass had changed for the worse but clover had maintained its good condition. A very slight rise above the critical value then occurred, the plants remaining without noticeable change, and the supplying power decreased again to below the critical value by September 22. On that date blue-grass was very brown although clover was in good condition, but both plants showed bad effects of drouth four days later.

In a similar manner the relation between rises of the soil-moisture graph from below to above the critical value 100 may be studied with reference to improvement in the condition of the plants. Without going further into such details in this discussion, however, it may be said that whenever the soil-moisture index remained below the critical value for as much as four or five days a definite drouth effect was generally shown by both plants, unless, indeed, they were already in very bad condition. Blue-grass often responded more promptly than clover in these cases. Injury, or increased injury, may be noted in a shorter period than four or five days and was sometimes apparent to some extent even by the time the index value had descended to 100. Similarly, beginning with apparently dead or badly injured plants, when the index value increased above 100 and remained there for a period of 4 or 5 days, the plants generally showed marked improvement by the end of that time. In such cases it was noted that clover improved more quickly than the other plant.

Somewhat superficial observations indicate that the plants of late summer, which had been subjected to a series of water deficiencies and had somewhat reduced leaf systems, were better able to withstand drouth than were those of early summer, which had been growing luxuriantly in the spring under very favorable conditions. The late summer drouth was more prolonged than was the drouth of early summer, although the latter was more severe for a short period. Also, evaporation was very intense for the early drouth and not nearly so intense for the later one, and this difference may partially account for the fact that the plants suffered more promptly and more intensely in the early summer than in the latter part of the season. MAXIMOW (17) has recently pointed out that repeated approach to wilting with corresponding recovery may render plants more drouth-resistant.

GENERAL CONCLUSIONS FROM THE SEASONAL STUDY

The results of this study of the Homewood lawn throughout the summer of 1925 make it clear that blue-grass and white clover responded definitely (although with some lag, as would be expected) to changes in the water-supplying power of the soil at the 6-cm. depth, as that soil feature is indicated by the soil-point method, especially when these changes occurred within the lower portion of the range of soil-point values, say below 500 or 600. Blue-grass seems to be particularly suitable as a plant indicator of soil-moisture conditions near the soil surface and white clover appears to be nearly as satisfactory. It seems hardly possible to question the conclusion that the main soil condition controlling the general vigor and color of a summer lawn such as the one studied is the water-supplying power of the soil and that soil-point determinations at the 6-cm. depth give very

useful numerical values for the effectiveness of this soil feature. It is clearly indicated that fluctuations in these values, especially for their lower ranges, were remarkably parallel with the concomitant changes in the general aspect of the plants. Attention should be directed again to the point that the higher soil-point readings are not to be rigorously compared among themselves and that they do not form a homogeneous and precisely commensurable series with the lower values. The higher values are useful as indicating periods when the moisture supply was amply adequate, when it was decreasing or when it increased. The influence of precipitation, as the climatic feature that mainly controlled the water-supplying power of the soil, is clearly brought out, and a secondary but notably independent influence of evaporation is indicated.

Turning to possible practical applications, it appears that the soil-point method as here employed offers a comparatively ready means for detecting the decrease of the water-supplying power toward and below the critical magnitude that is requisite for the maintenance of good color in a lawn such as the one here considered. Judging from the results of this study, it might be recommended that artificial irrigation be applied to this lawn whenever the supplying-power reading for the 6-cm. depth approaches the tentatively critical value of 100, as indicated by these soil-points. It seems safe to say that, for lawns of this sort and with the Baltimore summer climate, a satisfactory green color might be maintained throughout the growing season, as far as moisture supply is concerned, if irrigation were applied in such a way as to maintain in the soil at a depth of about 6 cm. a water-supplying power always above 100. Of course the water-supplying power will have very high values immediately after each separate irrigation, as is the case after each heavy shower, but it seems to be unnecessary to apply more water at any one time, if this general procedure is followed throughout the season, than is needed to bring the soil-point value to 500 or 600 for the 6-cm. depth on the day following the application of water. It may be that the employment of this method as a guide to the application of water might result not only in more satisfactory lawns but also in a considerable saving of water and of labor in applying it. From what is known about soil aeration in relation to plant growth it appears probable that the lawn plants might grow more vigorously if the soil at the 6-cm. depth were never allowed to approach its maximum field capacity, which corresponds, for the soil here studied and the soil-points used, to a soil-point reading of about 2000.

V. Studies of lawn slopes

THE HOMEWOOD BOWL

On rather steep slopes plants such as those here considered are usually more vigorous towards the base. As has been mentioned, even on an approximately level lawn the grasses sometimes appear greener in very slight hollows, this being probably due to surface run-off from the higher to the lower levels, and the same principle applies to slopes. A bowl-shaped area of lawn at the main entrance of the Homewood grounds of the Johns Hopkins University furnished an opportunity for studying the relation between slopes on the one hand and soil moisture and plant condition on the other. Observations for this purpose were begun early in June and continued until late in November, 1925, being carried on along with the other lawn studies here reported. The difference in level between the top and base of this slope is about 8 m. and the angle of the slope is about 30° in its steepest portion. The slanting part continues into an extended level area below, but the level area at the top is confined to a narrow strip less than a meter wide, bordering a curbed and paved driveway that lies along the rim of the Bowl, and the curb prevents water from above from overflowing on to the slope. This part of the study was made with reference to blue-grass, which is the dominant plant of the Bowl.

Soil-point determinations for the 6-cm. depth and observations on the condition of the grass were made on this area in the same manner as in the study of the level area reported above. These data were taken for three levels on the slope, namely: (1) at the top, just at the rim, where the level area above joins the slope below; (2) half way down the slope; and (3) at the base, on the level area just beyond where the soil surface becomes horizontal. The observations were made at varying intervals, the average time being about one week. No water was artificially supplied to any part of the slope.

The data secured are tabulated in table II and are shown in the form of graphs in figure 2. For the top of the slope the water-supplying power for each observation is given in column 2 of the table and these values are indicated by the continuous line in the upper part of the figure. The numerical scores representing the condition of the grass at the same level are given in column 5 of the table and are indicated by the continuous line in the lower part of the figure. Similarly, the values for successive water-supplying powers at the middle of the slope are given in column 3 of the table and are indicated in the upper part of the figure by the broken line, while the corresponding indices of grass condition are presented in column 6 of the table and by the broken line in the lower part of the figure. In like manner, the water-supplying-power values for the base of the slope are

shown in column 4 of the table and by the dotted line in the upper part of the figure, while the grass indices for the base are shown in column 7 of the table and by the dotted line in the lower part of the figure. Other data corresponding to these, especially with reference to rainfall and evaporation, may be referred to in table I and figure 1.

TABLE II

WATER-SUPPLYING POWER OF THE SOIL AND GROWTH CONDITION OF KENTUCKY BLUE-GRASS
AT THREE LEVELS ON THE SLOPING LAWN OF THE HOMEWOOD BOWL

DATE	WATER-SUPPLYING POWER OF SOIL (6-CM. DEPTH)			GROWTH CONDITION (SCORE VALUES)		
	TOP	MIDDLE	BASE	TOP	MIDDLE	BASE
June 2	9	26	45	0	2	3
5	5	13	43	0	1	2
10	6	16	15	0	1	2
20	7	10	19	0	0	1
July 3	8	17	17	0	0	0
8	16	27	29	0	0	0
16	58	302	448	0	0	1
28	176	362	481	1	2	3
Aug. 6	207	700	720	1	3	4
14	25	158	150	1	3	4
19	10	40	95	0	2	3
26	10	35	44	0	2	3
Sept. 1	135	200	442	0	1	3
4	12	30	235	0	1	2
10	13	40	83	0	1	2
18	35	230	530	1	2	3
25	14	33	51	0	2	3
Oct. 1	15	27	47	0	1	2
5	64	93	450	1	2	3
12	75	575	637	1	2	3
21	105	279	325	1	3	3
30	655	1460	1535	2	3	4
Nov. 10	910	1502	1730	1	2	3
19	1415	1993	2046	1	2	3

The strip of lawn along the Bowl rim was conspicuous throughout the season, for the grass was always either apparently dead or of very poor color. Slight greening (index value 1) occurred for short periods at several times during the season, but never to any great extent. Scattered plants of narrow-leaved plantain (*Plantago lanceolata* L.), a very drouth-resistant form, showed marked retardation or drouth injury throughout most of the season in this marginal strip at the top of the slope. After the end of the first summer drouth each slight, temporary recovery of the grass corre-

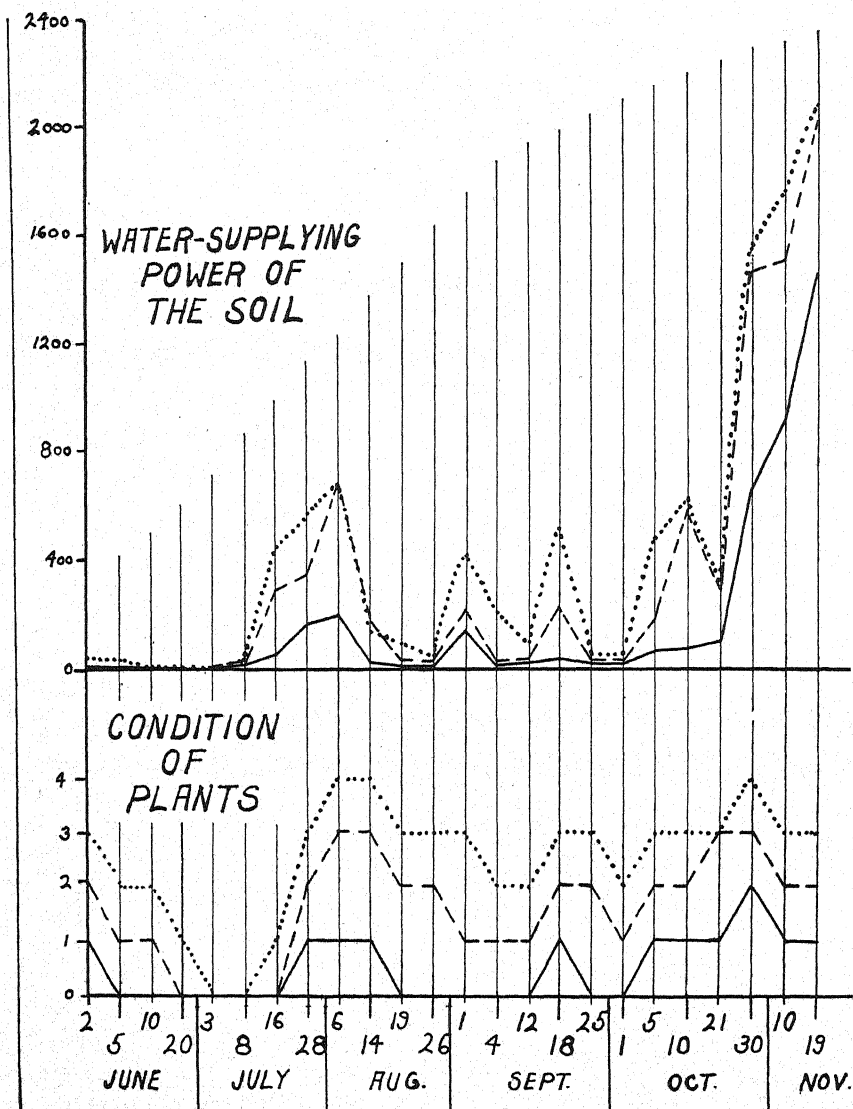


FIG. 2. Graphs of data given in table II, for the sloping lawn at Homewood, showing water-supplying-power values (upper part of figure) at intervals from June to November and corresponding scores (lower part of figure) representing the condition of Kentucky blue-grass. Full line represents top, broken line represents middle, and dotted line represents base of slope.

sponded in time to a temporary increase in the water-supplying power of the soil, the value of which was, however, very low throughout the entire period until late in October. Most of the time it was below the critical value (100) brought out in the preceding section and when it rose above that value the rise was only temporary.

After the close of the severe drouth of June and early July, when the grass on all parts of the slope had the appearance of being completely dead, the grass of the middle region generally showed better color than was shown at the top, but its condition was never better than "good." Correspondingly the water-supplying power was always somewhat greater for the middle than for the top of the slope throughout the period following the early summer drouth, and a clear relation is generally shown between the fluctuations of the supplying-power values for the middle level and the fluctuations of the corresponding plant scores. After July 16 the water-supplying power here fell below the critical value for only three short periods. It was high for the latter part of July and the first part of August and again at the end of the season, after the middle of October. Corresponding to these last two periods, with the usual lag, the plant score attained the value 3, indicating good growth, the best observed for the middle level.

After the rains of early July the water-supplying power at the base of the slope was seldom below 100 for very long and, correspondingly, the grass color was good during most of the period from July 28 to November 19. It was excellent in the first half of August and again at the end of October. These are the only times when excellent growth was observed anywhere on this slope. It is also to be noted that the grass condition at the base was, throughout the season, always better than at the middle of the slope, with the exception of the drouth period of early July and the single observation of October 21, the middle and base of the slope being both recorded as good for the latter date. The decreasing and low water-supplying power of September 4 and 10 was accompanied by drouth injury (score 2). Then the slight rains of mid-September brought the color back to "good" for September 18 and 25, but injury was again apparent on October 1, corresponding to an exceptionally low value of the supplying power. Only four times after the close of the early summer drouth did the water-supplying power at the base of the slope fall below 100. In the first case (August 26) the deficiency in supplying power (below the critical value) was pronounced but it was apparently not sufficiently prolonged to cause considerable injury to the plants. In the second case (September 10) the soil index fell only slightly below the critical value and yet the grass showed notable injury. In the third case (September 25 and October 1) the soil-moisture index was well below the critical value for at least six days and the plants showed marked injury.

While the water-supplying power is generally shown as greater for the base of the slope than for the middle, it is to be noted that the values for these two levels are approximately the same when both are high. This is true for the observations of August 6 and October 12, although the maximum field capacity of the surface soil was not by any means approached at these times. At the end of the season (November 19) the three levels showed supplying powers in the order that would be expected, but the soil-point readings for the middle and base of the slope were nearly alike (1993 and 2046, respectively), while the rim gave a reading of only 1415.

None of the observations of this series showed the soil at the 6-cm. depth as approaching complete saturation; only at the end of the series was the maximum field capacity approximately reached, and then only for the middle and base of the slope. If it had been possible to make observations during or immediately after heavy rains it is probable that nearly complete saturation, with soil-point readings of about 3,500, might have been recorded, especially for the base. Even at this lowest level drainage was so good that nearly complete saturation was not maintained for more than an hour or two after the heaviest rains. On the other hand there was apparently little or no subterranean capillary movement of water down the slope, nor upward from deeper soil layers; the soil about the grass roots apparently received no water in significant amount excepting directly from precipitation and surface drainage. Consequently the water-supplying power fell to very low values even at the base of the slope when a rainless period was sufficiently prolonged.

The graphs of plant condition emphasize the tendency of the latter to lag behind the corresponding changes in water-supplying power. Also, differences between the three levels are especially noticeable with respect to the time required for the green color of the grass to disappear entirely with the advance of the early summer drouth. The grass was first recorded as all brown on June 5 for the top of the slope, on June 20 for the middle and on July 3 for the base. A similar relation between the three levels is shown, though somewhat less completely, for the second drouth period, of late August and early September.

Starting with the vegetatively dead grass of early July, similar differences between the three levels are to be observed with regard to grass recovery in response to the rain of July 7. For example, on July 28 the grass at the base of the slope had good color, that in the middle was well started toward recovery, while that at the top, although somewhat green, was still in very poor condition. After October 30 the grass at all three levels showed noticeable injury, although the water-supplying power of the soil was high in every case. The autumn injury was apparently due to low temperature.

SLOPES OF THE GOLF COURSE

Besides those on the Homewood Bowl, observations were also made on several slopes on the golf course of the Baltimore Country Club. This large tract lies two miles northwest of the Homewood grounds, the soil is like that at Homewood and the area is characterized by many long slopes, some of which are very steep. Four of these slopes (designated A to D) were studied in a manner similar to that followed for the Bowl and the data

TABLE III

WATER-SUPPLYING POWERS OF THE SOIL AND CORRESPONDING GROWTH CONDITIONS OF KENTUCKY BLUE-GRASS AND OF WHITE CLOVER AT THREE LEVELS ON FOUR DIFFERENT SLOPES OF THE GOLF COURSE OF THE BALTIMORE COUNTRY CLUB, SUMMER OF 1925

	DATE	WATER-SUPPLYING POWER OF SOIL (6-CM. DEPTH)			GROWTH CONDITION (SCORE VALUES)					
					BLUE-GRASS			WHITE CLOVER		
		TOP	MIDDLE	BASE	TOP	MIDDLE	BASE	TOP	MIDDLE	BASE
Slope D	July 17	38	49	415	0	0	3	1	1	4
	23	38	47	982	0	0	3	1	1	4
	Aug. 7	340	1050	1570	1	1	4	2	2	4
	10	50	73	1250	1	1	4	2	3	4
	18	30	36	660	1	1	4	2	3	4
	26	25	50	510	1	1	4	1	2	3
Slope C	Nov. 21	285	775	950	2	2	4	2	2	3
	Aug. 7	214	851	3083	1	3	4	2	3	4
	10	115	168	1945	2	3	4	2	3	4
	26	28	45	1323	1	2	4	2	2	4
	Aug. 7	468	1134	1930	0	3	4	1	2	4
	Aug. 7	85	925	1542	1	3	4

secured are presented in table III, which is arranged like table II. In this case the condition of white clover as well as that of blue-grass was recorded.

Slope A, the data for which are shown in the graph of figure 3, is a long slope with north exposure and about 20 per cent. inclination, the top being about 16 m. higher than the base. It continues at the top into a broad, nearly level area, from which surface drainage flows on to the slope itself. The main period of observation covered a period of about six weeks (from

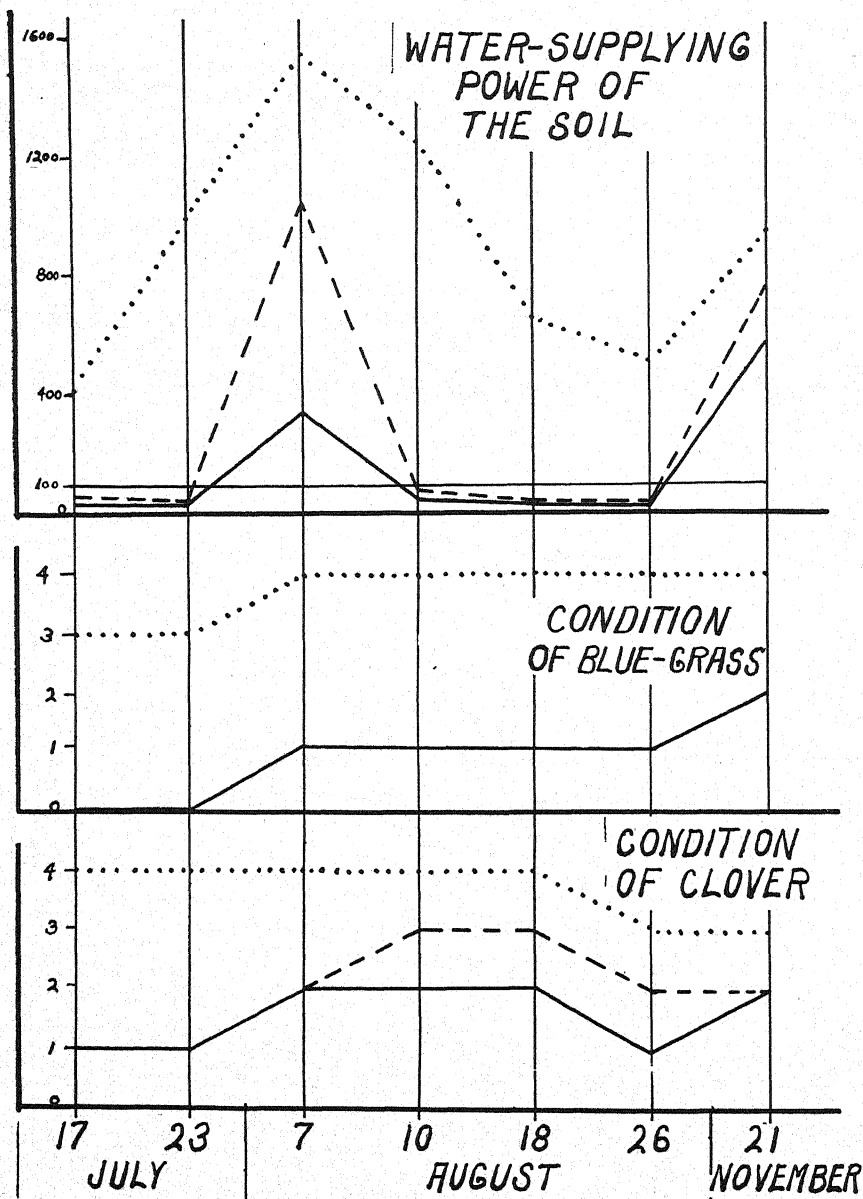


FIG. 3. Graphs of the water-supplying power of the soil (6-cm. depth) for base, middle and top of slope A, at the Baltimore Country Club, with corresponding data on the condition of Kentucky blue-grass and of white clover. Full line represents top, broken line represents middle and dotted line represents base of slope.

July 17 to August 26) and the results were in general much the same as for the Homewood Bowl.

The water-supplying power at the top of slope A was found to be above the critical value 100 only once in the period from July 17 to August 26, namely on August 7, and even then the value was relatively low. Neither blue-grass nor clover was recorded as in good condition at any time during the period. The rains of late July and early August improved the plant condition considerably but it was never very good.

At the middle of the slope the supplying power followed the corresponding value for the top throughout the period of observation, excepting that the value for the middle was much higher than that for the top on August 7. The condition of blue-grass was the same for both top and middle throughout the period. Clover, however, responded to the high water-supplying power about August 7 and this plant was in better condition at the middle than at the top for the remainder of the period. This may have been due to the penetration of clover roots into deeper soil layers than were reached by the blue-grass roots, for these deeper layers apparently maintained an adequate water-supplying power after that of the surface had fallen (August 10) to below the critical value. The similarity between the middle and top of this slope, with respect to both water-supplying power and plant condition, may probably be explained by reference to the surface run-off on to the slope from the area above. It appears that the soil at the top of the slope absorbed about the same amount of rainwater as was absorbed by the soil at the middle. It will be remembered that this sort of drainage from above did not occur in the case of the Homewood Bowl, because of the curbed drive at its rim.

At the base of slope A on the golf course the supplying-power index was greater than 400 at every test and much greater than 400 throughout most of the period. The plants of this level were in excellent or good condition for the whole period. Blue-grass was good at the start and it became excellent about August 7 and maintained that condition, while white clover was in excellent condition from the first observation until about August 26, when its condition seemed to deteriorate somewhat; on November 21 clover had nearly disappeared from this area, which was then almost completely occupied by very luxuriant blue-grass.

These observations for slope A of the golf course once more indicate that the water-supplying power of the surface layer of the soil needs to be above 100 in the Baltimore summer season if good or excellent growth of blue-grass and clover are to be expected.

Slope B also has a north exposure and it is otherwise much like slope A, excepting that there is an open ditch at its base. Three observations were

made here, on August 7, 10, and 26, as shown in table III. In all three cases the water-supplying power was found to be lowest at the top, considerably higher in the middle and very high at the base. Also the plants were generally in the best condition at the base and in very poor condition at the top, as in the case of slope A. On August 7, with a soil-point reading of 3083, it was observed that clover showed injury at the base of slope B, due probably to a deficiency in the oxygen-supplying powers of the soil (6), brought about by excess of soil water.

Slope C is long and gentle, about 200 m. long with a rise of about 20 m. It has a south exposure. On August 7 the plants showed marked differences in their condition at the top, middle and base of the slope. On that date the soil-point tests brought out corresponding differences in the water-supplying power of the surface layer of soil, as is shown in table III.

Slope D represents the head of an erosion gully in one of the hills of the golf course. The gully resembles the Homewood Bowl in the slope of its sides but is narrower and more V-shaped. On August 7 the difference between the plant condition along the upper margin and at the base was very marked, growth being excellent at the base of the V and very poor at the top, and corresponding differences in water-supplying power were indicated by the tests (see table III).

EAST LANSING SLOPE

Two series of observations on plant condition and water-supplying power were made June 18 and 22, 1925, upon a sloping portion of the campus of the Michigan State College, at East Lansing. This slope has a north exposure and rises about 7 m. in a distance of about 130 m. The soil is Miami loam, high in organic matter and well drained. On June 18, following a rain on June 17, the readings for the top, middle, and base of this slope were found to be 56, 131, and 1116, respectively. These readings illustrate once more the relation of water-supplying power to the different levels of a slope shortly after a rain. These values fell rapidly and on June 22 they were found to be 15, 20, and 305, respectively. At the time of the second observation white clover was dominant at the base and both it and what blue-grass was present at that level were in excellent condition. Clover was not present at the middle or top of the slope, but blue-grass was plentiful throughout most of the slope area excepting at the base. At the middle level blue-grass was in poor condition and it appeared practically dead at the top. Red fescue (*Festuca rubra* L.) was dominant at the top where it was in fair condition, being much more vigorous than blue-grass at that level. These observations suggest relations that probably exist in many cases between differences in water-supplying power and the local occurrence of different plant forms. On this East Lansing slope red fescue appeared

to be more xerophilous than blue-grass and blue-grass appeared more so than white clover.

SUMMARY OF RESULTS FOR SLOPE STUDIES

The slope studies reported above show that the water-supplying power of the soil at the 6-cm. depth was generally very different for different levels of the same slope, being lowest at the top and highest at the base. These differences were still evident even when rain had recently occurred. The configuration of the soil surface beyond the top of a slope seems to be important in determining how great will be the differences in supplying power for several levels on the slope. When water falling on the level beyond the top of the slope can flow over on the slope itself, the top may receive and absorb an extra supply, even as much as is absorbed at the middle, and the difference between the indices of water-supplying power for these levels may consequently be only slight after some rains. With the progress of a rainless period the supplying-power index decreases rapidly at the top of the slope and only slowly at the base. With prolonged and severe drouth the soil-point readings at all levels approached zero, thus tending to become nearly alike. The condition of the plants agreed in general with what was to be expected from the soil-point readings, being always much better at the base of a slope and often very poor at the top. The distribution of different plant forms over a slope appeared to be closely related to differences in water-supplying power for the different local areas; the more xerophilous forms are apt to be dominant in areas where the average supplying power is low or where it is especially low during drouth periods. Correspondingly, the least xerophilous forms usually dominate in areas in which the water-supplying power of the soil is never low for any long period. Of course it is to be remembered that the studies reported in this paper dealt with only the surface layer of the soil, to a depth of about 6 cm. Deeper soil layers would, of course, need to be studied in order to bring out the relations of drouth resistance for more deeply rooted forms. As has been said, blue-grass was especially responsive to alterations in the water-supplying power of the soil at the depth of 6 cm. Although blue-grass roots penetrate much more deeply, these 6-cm. determinations showed clearly and consistently the fluctuations in water supply that were reflected in alterations in the vigor of the grass, in the color of the leaves and in the general appearance of the turf.

VI. Miscellaneous lawn studies

While the observations and tests already presented were being made a number of soil-point readings were taken from time to time at special spots on the lawns, selected generally with reference to obvious differences in the

appearance of the plants. The results of these miscellaneous tests throw considerable light on various questions regarding the relation of the plant to the water-supplying power of the soil at the time. They are set forth below under several sub-headings.

SPOTS WITH EXCEPTIONALLY VIGOROUS PLANTS IN TIME OF DROUTH

At four different times in each of the two summer drouth periods some soil-point tests were made at selected spots on the lawns of the Charles Street boulevard (named Charles Street Avenue) adjacent to the Homewood grounds of the Johns Hopkins University, the spots selected being characterized by conspicuously good color and vigorous plant growth in the midst of a general lawn area that showed but little or no green color. Corresponding tests were made to determine approximately the water-supplying power of the general lawn area outside of the spots in question. While these boulevard lawns had blue-grass generally dominant, but in poor condition at the times of observation, the exceptional spots here considered showed other plants as very prominent or dominant and remarkably vigorous. Each of these tests refers to a special plant form, the following forms being included in the list: white clover (*Trifolium repens* L.), bermuda grass (*Capriola dactylon* Adans.), five-finger (*Potentilla monspeliensis* L.), yarrow (*Achillea millefolium* L.), blue-grass (*Poa pratensis* L.), bent grass (*Agrostis* sp.), crab grass (*Syntherisma sanguinalis* L.), and knotweed (*Polygonum aviculare* L.). The results of these tests are brought together in table IV.

TABLE IV

WATER-SUPPLYING POWER OF SOIL AT THE 6-CM. DEPTH FOR SPOTS WITH EXCEPTIONALLY VIGOROUS PLANTS IN TIME OF DROUTH, SUMMER OF 1925

PLANT FORM	JUNE 7	JUNE 10	JUNE 11	JULY 17	AUGUST 14	AUGUST 19	AUGUST 20	AUGUST 26
General lawn	12	10	19	25	103	37	50	65
Selected spots								
White clover	42	173	113	343	85	202
Crab grass	310	126
Five-finger	43
Bermuda grass	37	31	106	322	82
Yarrow	34	161	55	192
Blue-grass	189	221	145	115
Bent grass	116	80	215
Knotweed	283	60

It is clear that the water-supplying power of the soil at the 6-cm. depth was in every case considerably higher for the selected spot than for the general lawn at the same time. It does not necessarily follow, however, that the higher water-supplying power at the selected spot furnishes in every case the entire explanation for the exceptionally good growth. It must be remembered that these soil-point tests were made for a depth of only 6 cm. and it is probable that these exceptionally vigorous plants were absorbing water from much greater depths. This consideration has been mentioned in connection with the behavior of white clover in time of drouth. As to why the supplying power at the 6-cm. depth should have been uniformly greater in the selected spots than in the surrounding lawn, it may be that these spots sometimes received more rain-water than the rest of the lawn—on account of slight drainage on to them from surrounding areas or on account of their denser vegetation, which may have hindered surface drainage off from them. Also, if plants draw water mainly from the deeper soil layers the water supply of the surface layer may decrease much less rapidly during a dry period than would be the case if the plants drew water mainly from nearer the soil surface. The denser vegetation of these spots should retard direct evaporation from the soil surface, but this effect might or might not be counterbalanced by the larger leaf surface from which transpiration must occur. This would be determined in any case by the degree of foliar resistance to transpiration characterizing the particular plant forms in question, as well as by the amount of foliage. It is clear from what has been said before that the higher water-supplying power shown for the blue-grass tests of this series offers a satisfactory explanation for the exceptionally good condition of this grass in the selected spots where it was dominant. It will be seen that the supplying-power values given for blue-grass in table IV are all well above the critical value 100, while the corresponding supplying-power values for the surrounding lawn are generally much lower.

COMPARISON OF SPOTS BEARING VIGOROUS PLANTS WITH ADJACENT SPOTS BEARING VEGETATIVELY DEAD PLANTS OF THE SAME KINDS

On different dates from June 11 to September 11 a measure of the water-supplying power was obtained at the same time for adjacent vigorous and brown spots on the Homewood lawns and along the Charles Street boulevard near Homewood. The spots were selected with reference only to the appearance of the plants and the aim was to secure evidence as to the relations between the soil-point readings corresponding to good and to very poor plant condition. The two spots compared in each case were dominated by the same plant species.

TABLE V

COMPARISONS OF SOIL-POINT READINGS MADE AT THE SAME TIME ON SPOTS BEARING VIGOROUS PLANTS AND ON ADJACENT SPOTS BEARING VEGETATIVELY DEAD PLANTS OF THE SAME KIND

DOMINANT PLANT	DATE	WATER-SUPPLYING POWER OF SOIL (6-CM. DEPTH)	
		FOR VIGOROUS PLANTS	FOR PLANTS WITHOUT GREEN LEAVES
Five-finger.....	Sept. 4	78	33
	11	72	27
Crab grass	Aug. 31	54	21
	Sept. 11	46	20
Bent grass	June 11	93	19
	July 3	45	15
	Sept. 4	65	23
Yarrow	June 9	45	15
	Aug. 31	58	19
	Sept. 4	60	22
	11	40	18
Bermuda grass.....	June 9	40	13
	Sept. 4	166	18
	11	94	16
Knotweed	Sept. 4	43	10
	11	25	8

The results of these comparisons are shown in table V, from which it will be seen that the water-supplying power of the soil beneath the vigorous plants was always much greater than that in the spots where the same kind of plants were brown and apparently dead. In only one case (knotweed, on September 11) was good growth accompanied by a soil-point reading lower than 40 and in only two other cases (yarrow, September 11, and crab grass, June 9) was it as low as 40. On the other hand, no soil-point reading from a "dead" spot was greater than 33 (five-finger, September 4) and only two were greater than 23 (five-finger, September 4 and 11). The lowest reading of the series is 8 (knotweed, September 11) and the next to the lowest is 10 (knotweed, September 4). It thus appears that knotweed is to be considered as the most drouth resistant of the 6 plants here studied, as far as the water-supplying power of the soil at the 6-cm. depth is concerned. Five-finger is to be regarded as the least resistant of these plants. The other forms of this list are apparently intermediate in this respect. It must be emphasized again that these soil-point determinations were all for the

6-cm. depth. All of the plants of this series surely draw water from much greater depths.

RELATIVE DROUTH RESISTANCE OF DIFFERENT KINDS OF PLANTS, AS INDICATED
BY SOIL-POINT READINGS CORRESPONDING TO VERY POOR
GROWTH IN DRY PERIODS

Determinations of the water-supplying power of the soil were made, from time to time in dry periods, at spots in these lawns where selected plant species were in very bad condition, with about one-half of the leaves dead. Times and places were so chosen that the spots tested should be always characterized by apparently about the same degree of drouth injury. Of course this could not be done very accurately but the results may serve at least as indications of the approximate values of the initial water-supplying power at the 6-cm. depth when these selected plant forms became badly injured, to a degree somewhat like that called "permanent wilting" by BRIGGS and SHANTZ.

Ten species that occurred commonly, but some of them locally, on these lawns were selected for this series of tests and several observations were made for each species. The several values secured for each form have been averaged in each case and the averages are shown below, the species being arranged in the descending order of the magnitudes of the mean water-supplying power corresponding to the particular degree of drouth injury on which these observations were based.

Names of plants	Water-supplying power (6-cm. depth)
Blue-grass (<i>Poa pratensis</i> L.).....	39
Bent grass (<i>Agrostis stolonifera</i> L.).....	33
Crab-grass (<i>Syntherisma sanguinalis</i> L.).....	32
Five-finger (<i>Potentilla monspeliensis</i> L.).....	30
White clover (<i>Trifolium repens</i> L.).....	29
Yarrow (<i>Achillea millefolium</i> L.).....	23
Bermuda grass (<i>Capriola dactylon</i> Adans.).....	19
Red fescue (<i>Festuca rubra</i> L.).....	13
Narrow-leaved plantain (<i>Plantago lanceolata</i> L.).....	11
Knotweed (<i>Polygonum aviculare</i> L.).....	9

It will be seen that the value of the supplying-power index ranges from 39 for blue-grass to 9 for knotweed. The rather low degree of precision with which these values were determined may render it undesirable to attempt any very detailed considerations of the exact positions of the several plants in the series, but it is worth while to note at least that the first three species have values lying within the upper one-fourth of the entire range,

while the values for the last three species all lie within the lowest one-fourth. Whatever may be the case in regard to the four plants occupying the middle portion of the list, it may be safe to say that blue-grass, bent grass, and crab-grass showed the lowest drouth resistance and that red fescue, narrow-leaved plantain and knotweed showed the highest resistance.

The results of this preliminary and somewhat superficial attempt to classify a number of plant forms on the basis of their ability to withstand soil drouth make it clear that marked differences are to be expected between different species, growing under the same climatic conditions and in the same kind of soil. One of the main difficulties in attempting the classification of a number of different plants, with reference to their ability to withstand low rates of water-supplying power in the soil about their roots, seems to lie in determining just when a given plant individual has reached the particular degree or stage of drouth injury that is chosen as the basis of the classification. If it were possible to be sure that the plants were all at the same physiological stage of drouth injury when the soil-point tests were made, then the detailed results of such a series of comparisons as the one here attempted would surely be more reliable, especially if determinations for greater soil depths were also included. From experience gained in the present studies it seems safe to say that the method of determining water-supplying powers of the soil by means of porous-porcelain soil points gives results of a higher degree of precision than can yet be reached in determining the exact degree of drouth injury in a plant or group of plants.

PROGRESSIVE LOWERING OF THE WATER-SUPPLYING POWER OF THE SOIL AS THE
LEAVES OF WHITE CLOVER AND BLUE-GRASS WILTED,
WITHERED, AND FINALLY DIED

Beginning early in the summer drouth, soil-point readings were taken regularly at two-day intervals, at spots where white clover or blue-grass was at first in excellent condition, the readings being continued until the leaves of the plants were all or nearly all dead from drouth effect. This series of decreasing values of the water-supplying power of the soil at the 6-cm. depth, accompanied by corresponding notes with regard to the apparent condition of the plants, is interesting as showing how the foliage showed increasing drouth effect and finally succumbed with the progress of a drouth period.

On June 2 the average soil-plant reading for clover in excellent condition was 184, while good areas of blue-grass gave readings averaging 40. On June 4 clover was vigorous, being rated as good, with a water-supplying power of 131, and blue-grass was noticeably wilting with a supplying power of 24. On June 6 clover was beginning to show the effect of water deficiency and the grass was very badly wilted and losing color, the corresponding

values for the water-supplying power being 76 and 18, respectively. On June 8, with a water-supplying power of 54, clover was badly wilted and many leaves were dead, while with a water-supplying power of 12, the leaves of blue-grass were all dead and no green color remained.

It appears that white clover passed from the vigorous condition to a condition with many of its leaves dead in a period of 6 days, during which the corresponding index of water-supplying power decreased from 184 to 54. In a similar way, and in the same time, blue-grass changed from the good to the vegetatively dead condition, while the corresponding index of water-supplying power decreased from 40 to 12. It is noticeable that each of the supplying-power values, including the first in each series, is much greater for clover than for blue-grass. This is partly to be explained by the facts that clover was in somewhat better relative condition at the beginning of the series (June 2) than was the other plant, and that a like relation still held at the end of the series (June 8). Also, as has been mentioned before, spots on which clover was dominant and vigorous generally showed somewhat higher water-supplying powers for the 6-cm. depth than did adjacent spots with dominant and vigorous blue-grass.

From other evidence presented in the foregoing pages, clover is to be regarded as more drouth resistant than blue-grass, with reference to the soil-point determinations as here made. This statement seems to be contradicted by the set of data just given, but, besides what has been said in the last paragraph, it must be remembered that it requires considerable time for the plants to respond to a given soil condition after the latter has been reached. The observations here considered were made in the early part of a drouth period, at a time when environmental aridity was very rapidly

TABLE VI

SOIL-POINT READINGS (6-CM. DEPTH) TAKEN AT TWO-DAY INTERVALS DURING THE EARLY PART OF A RAINLESS PERIOD IN WHICH WHITE CLOVER AND BLUE-GRASS PASSED FROM EXCELLENT OR GOOD CONDITION TO VERY POOR CONDITION

DATE	WHITE CLOVER		BLUE-GRASS	
	INITIAL WATER-SUPPLYING POWER OF SOIL	GROWTH CONDITION	INITIAL WATER-SUPPLYING POWER OF SOIL	GROWTH CONDITION
June 2	184	Excellent	40	Good
4	131	Good	24	Wilting
6	76	Wilting	18	Becoming brown
8	54	Becoming brown	12	Dead

increasing. It may be that any note on plant condition given in this series may correspond to the next preceding or even to an earlier soil-point reading, the physiological effect of the progressive decrease in water-supplying power always showing a marked lag behind the decrease itself.

On account of their special interest and because of the somewhat complex causal relations that they suggest these particular data are summarized in table VI.

PLANTS IN DIFFERENT DEGREES OF HEALTH IN CLOSELY ADJACENT SPOTS
ON THE SAME LAWN

It was noticed at several times that blue-grass and clover on some spots or small areas appeared to be in very different condition from that of the same kind of plants elsewhere in the lawn. Some of these cases were specially studied, since they offered an opportunity to relate differences in plant condition to corresponding differences in the water-supplying power of the soil at the 6-cm. depth. The supplying power itself may, for the same precipitation, be related to the kind of soil, whether more or less sandy, etc., and to the surface and subterranean drainage conditions, as well as to the previous withdrawal of water by plant roots. Of course there are many soil conditions besides water-supplying power that may enter into the explanation of local differences in vegetation, such as: degree of compactness of the surface layers (resulting in differences in penetrability toward rain-water and toward growing roots), concentrations of soluble chemical constituents in the soil solution, number and kinds of soil organisms, and mechanical or other injuries to which the plants themselves may have been subjected. However, for the spots here studied the health of the plants was apparently definitely related in every case to differences in the water-supplying power of the soil as indicated by the soil-point method. In many of the cases tested the places compared were less than 2 m. apart.

Table VII gives some of the data from these observations on particular spots in the lawns at the Baltimore Country Club and at Homewood. The first section of the table presents observations on blue-grass for the period of the second drouth of the summer, and the third section gives data from October and November observations on blue-grass spots that had appeared dead in August and September, in comparison with data from corresponding observations on spots where the grass had been good or excellent during the last-named months. The second section of the table presents observations on white clover at the time of the first and second summer drouth periods. Throughout the table two average soil-point readings are given for each date (in two cases three are given) and the condition of the plants at the time of observation is shown by the placing of the supplying-power in-

dices in the last four columns. Four degrees of health are here considered: excellent, good, injured, and dead; conditions 1 and 2 of the former discussions are here combined as "injured."

TABLE VII

DATA FROM SIMULTANEOUS SOIL-POINT TESTS IN DIFFERENT BUT CLOSELY ADJACENT PLACES THAT SHOWED DIFFERENCES IN CONDITION OF BLUE-GRASS AND OF WHITE CLOVER

	DATE OF OBSERVATION	GROWTH CONDITION OF PLANTS AND AVERAGE SOIL-POINT READINGS			
		EXCELLENT	GOOD	INJURED	DEAD
Blue-grass in period of second summer drouth	Aug. 10	605	20
	18	712	30
	18	80	15
	26	182	22
	26	105	10
	31	78	23	10
	Sept. 4	300	15
	11	65	12
	18	985	32
	25	101	19
White clover in period of second summer drouth	June 8	60	16
	10	195	15
	11	46	19	8
	Aug. 31	50	17
	Sept. 11	230	36
Blue-grass after the rains of October	Oct. 5	707 ^a	31 ^b
	12	1105 ^a	60 ^b
	21	808 ^a	156 ^b
	26	1403 ^a	387 ^b
	30	1482 ^a	433 ^b
	Nov. 19	2134 ^a 1514 ^b

^a Grass good in September.

^b Grass dead in September.

Examining the first section of table VII, it is noticed that with supplying-power values of 65 or below the grass is always recorded as injured or dead and that for all values below 23 it is shown as dead. Also the grass plants are shown to have been in good or excellent condition for supplying-power readings of 78 or higher. These results agree in general with those already given and support the idea that blue-grass, with this soil and climate, may not be expected to be in satisfactory condition unless the water-supplying power of the soil has an index value not much below 100. Of

course, this critical limit may sometimes appear to be somewhat lower for a few days in the early part of a drouth period, when the water-supplying power is decreasing rapidly but the grass has not yet visibly responded to inadequate water supply.

Consistent with indications from other results in this study, white clover is again shown by the data of table VII to be apparently considerably more vegetatively drouth resistant than is blue-grass; in these observations this clover is recorded as in good condition with a supplying-power index for the 6-cm. depth as low as 46 (the corresponding limit for blue-grass being 78). As has been said, this difference may possibly be due to deeper penetration of the clover roots into the soil.

The remaining observations on blue-grass (see the last section of table VII) were made after the rains of early October had broken the prolonged drouth of August and September. These tests were made in places of two sorts, (*a*) where the grass had been in good condition at the beginning of the October rains, not having been significantly injured by the drouth, and (*b*) where the grass appeared dead at the beginning of these rains, having been greatly injured by the preceding drouth. The superscript letters *a* and *b* in the table indicate observations from these two kinds of areas, respectively. Each observation shown in the table represents an average of three tests of four soil-points each at different but like spots in the lawn, and thus the values marked *a* represent the general condition, at the time of observation, of areas that had not been previously injured by the drouth, while those marked *b* represent the general condition, when these tests were made, of the previously drouth-injured areas.

The data of this section show the manner in which the grass on spots that appeared dead at the end of the drouth revived and became progressively better with increasing water-supplying power of the soil after the rains began. The water-supplying power of the drouth-injured areas increased progressively from 31, on October 5, to 1514, on November 19, and in the meantime the grass improved from being apparently dead before the October rains began to the good condition indicated for November 19.

These data show also how the water-supplying power of originally dead areas increased much more slowly and gradually, but at an increasing rate, than did the supplying power of areas originally in good condition. This may indicate less absorption of rain-water by the soil of the originally dead areas, due perhaps to scanty cover, slight elevation above adjacent areas, or less permeability of the soil surface.

WATER-SUPPLYING POWER OF THE SOIL AT THE SIX-CENTIMETER DEPTH IN THE VICINITY OF TREES

The shading effect of trees upon the growth of lawn grasses in the soil area underneath their branches is often noticed. Other features besides

shading itself, such as modifications of rainfall, probably take part in producing apparent differences between the shaded and unshaded areas, and the influence of tree roots (which generally remove water at a rapid rate from the deeper soil layers) may sometimes be important. Of course the kind and degree of shading (that is, the modification of sunshine and diffuse-light conditions) determines whether it will be beneficial to the grass or not. In a drouth period the right degree of shading is generally of advantage to the lawn plants, since the shade retards both evaporation from the soil surface and transpiration from the leaves. At such times a marked beneficial influence was shown in many instances of these studies, the grass being in much better condition over most of the soil area underneath the tree branches, especially on the northeast side of the trunk. It is of course possible for the shade of a large tree or group of trees to be so complete that most species of lawn grasses are entirely eliminated in the most densely shaded area, probably because the light intensity is there too low for adequate photosynthetic activity.

During the progress of these lawn studies a number of soil-point tests were made at Baltimore and also at East Lansing, Michigan, to secure information about possible differences in the water-supplying power of the soil at the 6-cm. depth for the vicinity of trees and for surrounding unshaded areas. Cases were selected in which the lawn plants showed marked differences in their growth condition.

On June 23, at a distance of 1.5 m. from the trunk and on the south side of a large sugar-maple tree at East Lansing, the index of water-supplying power at a depth of 6 cm., derived as the average from four soil-point tests, was found to be 238, with blue-grass in excellent condition; while 8 m. farther from the tree and on the same side (well beyond the shaded area) the index value was only 68, with blue-grass similarly plentiful but in very poor condition, the leaves being almost all dead. Still farther away from the tree, in a representative part of the general lawn area, the index value was 76, with blue-grass in poor condition. In another unshaded spot on the same lawn red fescue was in good condition, however, with an index value of 24. About 1.5 m. southeast of the trunk of a large oak tree in the same lawn the water-supplying power at a depth of 6-cm. was found to be 85, with blue-grass in good condition, while soil-point tests made just beyond the spread of the branches of this tree gave a mean value of 53, blue-grass being here in very poor condition. In the case of the maple the lower branches were only about 3 m. from the ground and the crown was very dense, effectively cutting off practically all direct sunshine. The crown of the oak, on the other hand, was much more open and its lowest branches were about 12 m. above the ground; consequently the shade cast by this tree was much less complete than that cast by the maple.

On July 17, about 3 m. east of the trunk of a large ash tree on the golf course of the Baltimore Country Club, blue-grass was in excellent condition, with a mean soil-point reading of 292 at a depth of 6 cm. Four meters farther east, beyond the shading influence of the tree, blue-grass appeared poor and the mean soil-point reading was 38. Other tests on the same part of the lawn but well away from any trees gave an average water-supplying power of 43, the condition of blue-grass being generally poor.

A large beech tree in Wyman Park, which is adjacent to the Homewood grounds of the Johns Hopkins University, furnished an example of exceptionally dense shade. On the north side of this tree there was no grass and practically no vegetation at all for a distance of several meters out from the base of the trunk. Tests in this grassless area 1.5 m. from the trunk and at the 6-cm. depth gave a water-supplying power of 282 on August 19. Five meters east of this tree, still in the shaded area, blue-grass was excellent with a supplying-power reading of 782, and at the same distance to the north the condition of the grass was just as good, with a water-supplying power of 489. Tests made well outside the shaded area, where blue-grass was generally in only fair condition, gave an average soil-point reading of 78.

An excellent example of the beneficial influence of moderate shade on the health of grass was noticed throughout the summer in the case of a *Liriodendron* tree on the Homewood grounds. Blue-grass was healthy and vigorous on the northeast side of this tree, even during the worst drouth periods, when it appeared dead on the south side and on the surrounding lawn. On August 30 the supplying-power reading was 89 for the northeast portion of the shaded area while the general condition of the lawn was represented by a water-supplying index of 26. On September 11 a similar relation was observed, with a supplying-power reading of 415, in the northeast portion of the shaded area, while the reading for the lawn in general was 40. On both these dates the grass was growing well in the shaded area on the northeast side of the tree, while it was only slightly green on the south side, even under the branches, and over most of the lawn. Of course the summer shade produced by a tree in this latitude is much more effective northeast and north of the tree than south and southwest.

APPARENT INFLUENCE OF MOWING THE LAWN ON THE WATER-SUPPLYING POWER OF THE SOIL AT THE SIX-CENTIMETER DEPTH

At several times during the season simultaneous soil-point readings at the 6-cm. depth were compared for mowed and unmowed portions of the golf course previously mentioned. In all these cases the grass was in fair or good condition at the time of the tests and the results show very clearly that the supplying power was markedly lower in the mowed areas at the

time of each observation. This feature was not studied sufficiently to warrant any attempt to explain these findings, although it is not difficult to think of plausible explanations, and the data are recorded here (table VIII) simply as empirical observations.

TABLE VIII

COMPARATIVE WATER-SUPPLYING-POWER VALUES AT THE 6-CM. DEPTH FOR MOWED AND UNMOWED AREAS OF LAWN

DATE OF OBSERVATION		MOWED AREAS	UNMOWED AREAS
August	10	328	437
	18	20	75
	18	18	70
	26	26	170
November	21	820	1550

RELATION OF THE SOIL-MOISTURE INDEX TO THE COMING-UP OF FIELD MUSHROOMS ON THE HOMEWOOD LAWN

The field mushroom (*Agaricus campestris* L.), one of the most common of the edible fall mushrooms of the Baltimore region, usually forces its fruiting bodies above the soil surface in late August and September. These months were unusually dry in 1925 and the appearance of this mushroom was considerably delayed. However, many small groups of *Agaricus* appeared September 4, four days after the drouth-breaking rain of August 31. These groups were generally confined to spots on the lawn where blue-grass was in good condition, although the blue-grass of most of the lawn appeared partly brown at that time. This suggested that the coming-up of the mushrooms might be closely related to soil-moisture conditions.

On September 4 ten soil tests were made in groups of mushrooms on the level portion of the Homewood lawn, where blue-grass was vigorous. The average index of water-supplying power for the 6-cm. depth was 170. Other areas of good blue-grass, but without mushrooms, gave an average index value of 142. Still other areas with poor blue-grass and also without mushrooms gave an average index value of only 36.

Other determinations made on the same date for groups of mushrooms with vigorous blue-grass present, near the base of the Bowl slope at the entrance of the Homewood grounds, gave an average water-supplying power of 138. The corresponding average from tests made at the base of this slope, where blue-grass was in poor condition and where there were no mushrooms, was only 38.

These data clearly indicate the existence of a relation between the water-supplying power of the soil at the 6-cm. depth and the coming-up of these

mushrooms, and they suggest that the critical water-supplying power for the appearance of these fruiting bodies above the soil may be at least 100, the same value as has been suggested as critical for vigorous growth of blue-grass and white clover. Of course, it is understood that the earlier development of *Agaricus* proceeds in the soil for a long time before the fruiting bodies appear above the surface. Whatever may be the necessary moisture conditions for the mycelial growth and the original formation of the young sporophores, it seems clear that these emerged from the soil in the case studied only when the water-supplying power of the soil was such as to give vigorous growth of blue-grass. The temperature and other non-water relations for mushroom development may be very different from those for the growth of blue-grass, and mushrooms would not be expected to appear excepting at their proper season, even though the moisture condition of the soil might be quite suitable.

WATER-SUPPLYING POWER OF THE SOIL AT DIFFERENT DEPTHS

As has been said, all of the soil-point tests thus far considered refer to the surface layer of the soil, the instruments being so placed that the middle of the absorbing zone of the porcelain cone was in each case about 6 cm. below the soil surface. While most of the absorbing roots of blue-grass seemed to occupy the soil to a depth not much greater than that at which the instruments were set, and while this grass responded very quickly and definitely to changes in the water-supplying power at the 6-cm. depth, yet it is safe to suppose that some of the blue-grass roots drew water from deeper soil layers, and this consideration is still more applicable to the case of white clover and the other plants mentioned in these special studies. It has been mentioned that this clover, as well as yarrow, knotweed, etc., and other grasses than blue-grass, responded less quickly than did blue-grass to moisture changes at the 6-cm. depth, probably because of the deeper position of many of the roots. On account of such considerations as these it was especially interesting to inquire, in a preliminary way, as to how the water-supplying power of the soil might vary with increasing depth.

It was not generally feasible in these studies to consider any other depth than the usual one, but a single series of observations at two additional depths was carried out on July 15 in an area of the Homewood lawn where blue-grass was in very poor condition and white clover was showing marked evidence of drouth injury. Six holes were dug in the lawn, three to a depth of 15 cm., and three to a depth of 30 cm., the soil at the bottom of each hole being left undisturbed. Two soil-points were placed in the usual way at the bottom of each hole. Six instruments were thus used for a depth of about 21 cm. below the general surface of the ground, and six more were used for a depth of about 36 cm. At the same time six other instruments were em-

ployed to determine the water-supplying power at the usual 6-cm. depth in the immediate vicinity. The three sets of six readings gave the following averages:

<i>6-cm. depth</i>	<i>21-cm. depth</i>	<i>36-cm. depth</i>
34	60	172

It is seen that the water-supplying power of the soil was greater with greater depth, at least to a total depth of 36 cm., which would be expected in a dry period.

A slight rain coming after a long dry period may raise the water-supplying power of the surface soil without greatly altering that of the deeper layers, and shortly after the occurrence of such a rain the supplying power of the surface layer may be considerably higher than that for a greater depth. Such a reversal in moisture conditions is probably not infrequent in many arid regions and generally in dry periods with infrequent showers. Going downward still farther the water-supplying power would probably be generally found to increase, finally becoming practically infinite when the subterranean water-table is approached.

This general relation between soil-moisture conditions and depth was clearly shown by some tests made on July 12, in very light, sandy soil near the Magothy River, south of Baltimore. Three tests were made within an hour after the end of a light rain, when the upper few centimeters of the soil were very wet. The values obtained are as follows:

<i>6-cm. depth</i>	<i>21-cm. depth</i>	<i>36-cm. depth</i>
3470	2020	3397

Another comparison made in the same locality and a little later on the same day, but under the spreading branches of a pine tree and beneath a dense layer of pine needles, gave the following values:

<i>6-cm. depth</i>	<i>36-cm. depth</i>
1702	728

If a thoroughgoing study of the ecological relations of soil-moisture conditions is to be made, it would be well to extend the supplying-power readings into the soil to a depth great enough to secure information on the water-supplying power of the soil adjacent to all important portions of the plant root systems dealt with. To work out the ecological soil-moisture relations of any kind of plant it will of course be necessary to take into account not only the variations in water-supplying power with different depths but also the configuration of the root systems considered, and especially the different depths and depth ranges from which the plant in question derives its

water supply. To carry out such a project would naturally involve very much more work on the part of the investigator than could be devoted to the present preliminary and reconnaissance study. Indeed, such a thorough study as has just been suggested would generally require the cooperation of several workers. Nevertheless, such studies should be much more readily carried out by means of the soil-point method than by any other means thus far introduced.

SOIL-MOISTURE CONDITIONS OF A THOROUGHLY WATERED LAWN

A number of soil-point tests were made, at several times in the growing season of 1925, on one of the very well kept greens of the golf course of the Baltimore Country Club, and simultaneously on the less well kept border of the green and on the surrounding lawn. The sod of the green was composed of an exceedingly dense growth of Washington bent grass, artificially watered every day excepting in periods of rain and mowed almost daily, with the mower knife set very low so that the grass never attained a height much greater than about two centimeters. The border, about 1.5 m. wide, was occupied by blue-grass, which probably received somewhat less artificial watering and was mowed less frequently and not so closely. The readings representing the surrounding lawn were made about 5 m. out from the edge of the green itself, where very much less water was added artificially than in the case of the green and border and where blue-grass was still dominant but not nearly as luxuriant as in the border.

The water-supplying-power values that were secured, for the 6-cm. depth, are shown in table IX. The exceptionally high values for all three tests on July 23 are due to an efficient rain that occurred on the preceding day.

TABLE IX

COMPARATIVE WATER-SUPPLYING-POWER VALUES AT THE 6-CM. DEPTH OF A WELL-WATERED GOLF GREEN AND THE SURROUNDING LAWN

DATE	GOLF GREEN	BORDER	SURROUNDING LAWN
July 17	1027	465	115
23	2652	2040	1271
Aug. 10	1452	1355	230
Nov. 19	1813	1692	523

These observations indicate that where great care was taken of a lawn area, particularly in the case of a golf green, the artificial irrigation (applied according to the judgment of the greenskeeper) resulted in maintaining the water-supplying power of the soil of the surface layer at a value not lower than about 1,000. It seems probable that the green here considered

was artificially watered somewhat more than was actually necessary, but good practice would generally apply some excess. On the other hand, there is, of course, some danger in too excessive irrigation, which might reduce soil aeration—the oxygen-supplying power of the soil; see HUTCHINS and LIVINGSTON (7) and HUTCHINS (6)—to a degree that would be directly or indirectly injurious to the grass, and might also encourage attacks on the grass roots and leaves by parasitic fungi, etc.

Summary

The two most important influences of the environment acting on upland plants during the growing season, as far as the water relations of such plants are involved, are the water-supplying power of the soil about their roots and the evaporating power of the air about their leaves. The water-supplying power of the soil is considered in some detail in this paper, with special reference to lawn plants, and some attention is given to evaporation, especially to the relations between the two.

Soil-moisture conditions were studied by means of the soil-point method, which involves the use of small, porous-porcelain cones to absorb water from the soil about the plant roots. The ability of the soil to deliver water to the porous-porcelain absorbing surface placed in capillary contact with it is considered as a measure of its initial ability to deliver water to a surface unit of the plant root system. The soil-point readings are expressed as the amount of water, in milligrams, absorbed by a single soil-point when exposed for one hour in the soil. The absorbing surface of the instrument is about 12 sq. cm. in extent.

Evaporation conditions were measured and compared by means of spherical, porous-porcelain atmometers, the amount of water lost, expressed in cubic centimeters, from the standard blackened sphere in a given period being considered as an approximate measure of the drying influence exerted by the aerial surroundings on the soil and plant.

A study was made of a Baltimore lawn with respect to the variations occurring in the water-supplying power of the soil at a depth of 6 cm., as these variations were related to the condition of the plants and to the intensity of evaporation, by which it was brought out that Kentucky blue-grass and white clover responded quickly and definitely to insufficiency of water when the water-supplying power of the gradually drying soil had decreased below a critical value and that these plants responded a little less quickly also to an increase in the water-supplying power when the inadequately wet soil was wetted by rain and the capacity of the soil to deliver water to the instrument increased from below to above the critical value. The plants responded in a few days (3 to 5) to variations occurring in the vicinity of

the critical value. Attention is called to the fact that high values of the water-supplying power, as given by this method, are not quantitatively comparable to values below about 600, but that values between 0 and about 600 appear to be comparable. The plants were healthy as long as the value of the water-supplying power of the soil was above about 500 and the critical value for the beginning of drouth injury, or for the beginning of recovery when the plants had been previously injured by an insufficiency in their water supply, was found to be about 100 or somewhat below. It seems that the soil-point method may be useful in determining and outlining the best irrigation practice, at least for lawns in humid or semi-humid, temperate regions. Its use might result in improved efficiency in the use of water, in the application of labor, and in better and more uniform grass condition throughout the growing season.

A series of studies on sloping lawn areas showed that the water-supplying power of the soil was generally highest at the base of the slope and lowest at the top, and that the plants were correspondingly most vigorous at the base and least healthy at the top. The distribution of different plant forms on a slope is apparently closely related to what would be expected from a knowledge of the variations in the soil-moisture index throughout the growing season, with the least xerophilous forms occurring or prevailing at points where the index value is never low for any extended time. The soil-point method consistently showed that the water-supplying power at the 6-cm. depth was generally notably higher in areas of healthy plants than in adjacent areas where the plants were visibly in poorer condition.

For periods of drouth in the shaded lawn area under the branches on the northeast side of a tree the lawn plants were generally much more vigorous than in the neighboring open lawn or on the southwest side of the same tree, and the soil-point readings showed corresponding differences in the water-supplying power of the soil.

Frequently mowed lawn areas generally gave considerably lower soil-point readings than did adjacent unmowed areas when the plants were in good condition in both areas.

In a lawn where several different plant forms occurred in local areas, each area being dominated by a particular form, observations on the health of the plants, taken together with corresponding soil-point readings made at the 6-cm. depth from time to time during the progress of a drouth period, indicated that these readings might serve as indices for classifying the several plant forms according to drouth resistance. Narrow-leaved plantain (*Plantago lanceolata* L.) and knotweed (*Polygonum aviculare* L.) were the most drouth-resistant forms noted in this way, while Kentucky blue-grass (*Poa pratensis* L.) was the least resistant. If the degree of drouth resistance

is estimated on the basis of soil-point readings taken in a drouth period and at the 6-cm. depth, it is of course to be expected that deep-rooted plants (such as white clover) will be indicated as more drouth-resistant than shallow-rooted plants (such as Kentucky blue-grass).

The common field mushroom (*Agaricus campestris* L.) appeared in these lawns only in spots where Kentucky blue-grass was in good condition and where the water-supplying power was above 100 at the time the fruiting-bodies were pushed above the soil surface.

It is possible to employ the soil-point method for studying moisture conditions at several depths. Some tests of this kind showed that the water-supplying power of the soil varied at different depths in a way that would be expected from a knowledge of recent precipitation and evaporation conditions.

The water-supplying power of the soil at the 6-cm. depth was found to be nearly always greater than 1,000 in lawn areas (as golf greens) where the grass was kept in excellent condition throughout the growing season by frequent watering and mowing.

For the range of values from about 2,000 to about 100 the quotient secured by dividing the decrease occurring in a given period by the total evaporation from the standard blackened atmometer sphere for the same period was found to be nearly constant, with a value of 4.3.

It is suggested that atmometer records kept during the growing season might give reliable indications as to when water should be applied to a lawn in order to keep it always in excellent condition.

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DIFFERENTIAL STAINING OF SPECIALIZED CELLS IN *BEGONIA* WITH INDICATORS

DEAN H. ROSE AND ANNIE M. HURD-KARRER

(WITH THREE FIGURES)

Introduction

Although it has long been known that *Begonia* plants are characterized by a very high oxalic acid content (8, 12), some recent reports of pH values for the expressed juice as low as 1.3 (22) and in some cases even lower (25) have again called attention to the high acidity of this plant. Values of this magnitude indicate hydrogen-ion concentrations almost as great as that of N/10 hydrochloric acid, and ten times as great as that of lemon juice (6, 10, 25). Acidities of approximately this magnitude have been reported for a number of fruits (1, 6, 9) in which the extremely acid sap is probably localized in special cells or vacuoles (9, 17). The reports of even higher concentrations of acid in leaf and stem tissue of *Begonia* raise the question as to whether the acid sap may be localized in special cells in this plant also.

Enzymes supposedly indispensable to plants are inactivated by acids of the concentrations found in the leaves and stems of *Begonia* (1, 3, 4, 7, 19, 26). Furthermore, it is now held (13, 20) that the normal hydrogen-ion concentrations of plant tissues are quite close to the isoelectric points of the constituent proteins. In fact, the pH values reported for most plants lie on the alkaline side of the isoelectric point (13, 20), and according to PEARSALL and PRIESTLEY (14), the isoelectric points of the principal plant proteins fall between pH 3.0 and pH 6.0.

There are statements in the literature, based on studies of the localization of acid in *Begonia* and other acid plants, to the effect that not all the cells are highly acid (8, p. 359, 12, p. 372). The observations noted in the present paper were made with the object of obtaining further evidence on this question by determining the color reactions of individual cells of freshly cut sections in indicator solutions.

Methods

The staining of freshly cut sections of plant material with solutions of various indicators has been quite widely used as a method of determining the acidity of individual cells (1, 15, 16, 24), although RUHLAND (21) questioned its usefulness with the indicators he used. It is recognized, of

course, that exact determinations of pH values are impossible with this method, owing to errors from interference of salts, proteins and other substances with the color reactions of the dyes. Nevertheless it was considered probable that for the purpose of the present investigation relative values might be obtained which would be significant.

The plants used in the investigation were, for the most part, *Begonia corallina lucerna* and *B. heracleifolia*.¹ They were grown in the greenhouse and studied during the winter months only.

Longitudinal sections and cross sections of petioles and young stems, and cross sections of the leaves were examined. The sections were cut fairly thin, from one to several cells thick. They were first rinsed quickly in water to remove the acid from the cut cells, and then placed either in several drops of the indicator solution on a microscope slide or in a larger quantity of dye in a depression of a drop-plate.

The indicators used were, for the most part, brom cresol green, brom phenol blue and thymol blue in aqueous solutions made up according to the recommendations of CLARK (5). Thymol blue and brom phenol blue were used in 0.4 per cent. solutions, but the brom cresol green was usually diluted to a 0.2 per cent. solution, and sometimes to 0.1 per cent.

For estimating the hydrogen-ion concentrations of the cells by their colors after staining, color standards were made up with buffer solutions covering the range of each indicator. The indicator chart given by CLARK (5) was also found useful for rough estimations.

Results

When sections of the stems or petioles of *Begonia* are immersed in a 0.2 per cent. solution of brom cresol green, they are immediately dyed yellow, indicating an acidity greater than pH 3.0. The originally green or blue external solution usually becomes yellow also, owing to diffusion of acid from the sections. If this acidified solution is removed and replaced with more of the original indicator solution, most of the cells turn green after 15 to 30 minutes, varying from yellowish green corresponding to about pH 4.4, which is frequently obtained with *B. heracleifolia*, to a blue green indicating about pH 4.8. With weaker indicator solutions, and relatively long immersions, these cells become a deep blue, corresponding to pH 5.0 or above. However, if the acidified indicator solution is not replaced by more of the original solution, the sections remain yellow for a long time, often for several hours, although they usually turn green or blue eventually.

¹ A few tests made on leaf petioles of ten varieties of *B. rex*, three of *B. metallica*, and one each of *B. prunifolia*, *peltata*, *ricinifolia*, *nelumbifolia*, *lucerna*, and *gilsonii* showed essentially the same staining reactions as those obtained with *B. corallina lucerna* and *heracleifolia*.

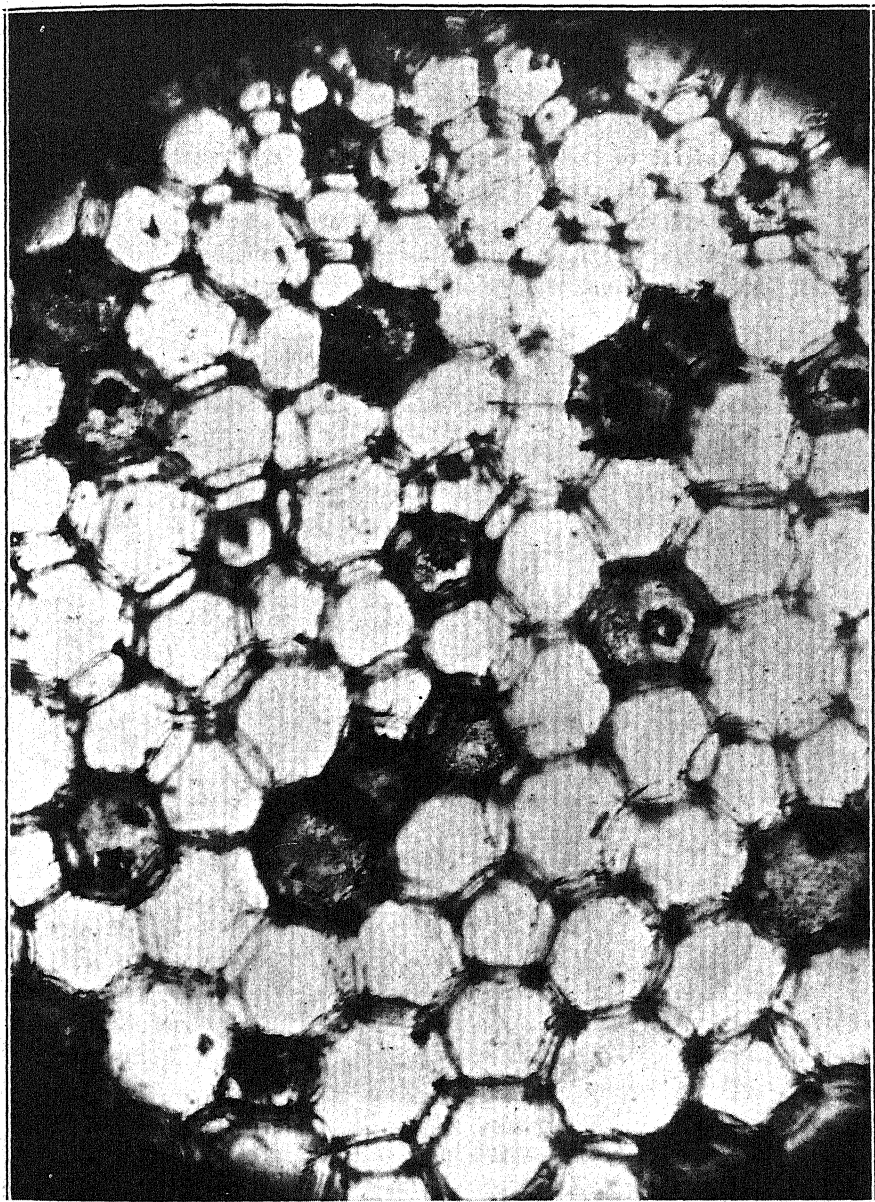


FIG. 1. Cross section of petiole of *Begonia heracleifolia* stained with brom cresol green showing distribution of specialized cells containing crystals of calcium oxalate.

Scattered without apparent order in cross sections, but appearing in rows in longitudinal sections are cells which do not turn green or blue on standing in brom cresol green (fig. 1). The yellow color taken on by these cells gradually deepens until after 15 to 30 minutes it becomes an orange yellow. After still longer intervals the cells become a reddish orange color. This intensification of the color suggests that the dye accumulates within the cells owing to interaction with proteins or other cell constituents (20). When stained under similar conditions with brom phenol blue, these cells become yellow ($< \text{pH } 2.5$), while the rest are purple ($> \text{pH } 4.4$); in thymol blue they become a brilliant red ($\text{pH } 1.2$ to 1.5),² while the rest

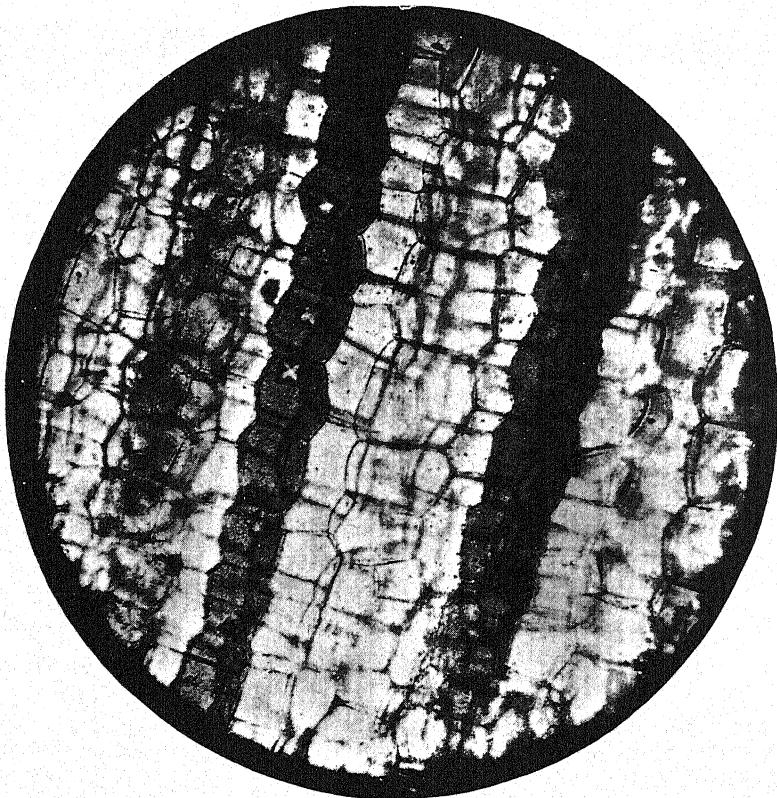


FIG. 2. Longitudinal section of petiole of *Begonia heracleifolia* stained with thymol blue showing distribution of specialized cells containing crystals of calcium oxalate. (Photograph of an autochrome).

² The specialized cells of *B. corallina lucerna* did not become red in thymol blue unless the sections had been previously treated with chloroform or ether, which apparently increased their permeability to the dye. With the other indicators this variety gave the same color reactions as *B. heracleifolia*.

are yellow ($> \text{pH } 3.0$). They contain crystals of calcium oxalate,³ and are evidently similar to the crystal-bearing cells reported to occur in a number of plants by DE BARY (2, p. 137), and others, and found by GIESSLER (8, p. 359), in *Begonia*. Thus, evidence of the specialized nature of these cells is afforded not only by the presence of the crystals but also by their staining reactions with indicators.

Occasionally, crystals were seen in cells that appeared from their staining reactions to be ordinary unspecialized cells; but in most sections they occurred almost entirely in the apparently specialized cells. In longitudinal sections the crystals were in rows (fig. 2) thus distinguishing the specialized cells even in unstained tissue (fig. 3).

It was found that the crystals predominating in *Begonia heracleifolia* were sometimes large tetragonal bipyramids (fig. 2), and at other times rosette aggregates (fig. 1). Those predominating in *B. corallina lucerna* were monoclinic forms occurring in rosette aggregates such as those shown for another species in fig. 3. PFEIFFER (15) has stated that the monoclinic form of calcium oxalate is precipitated from solutions having relatively high hydrogen-ion concentrations, while the other form occurs in neutral or basic solutions. Our observations do not substantiate this distinction so far as it applies to conditions in the cells of *Begonia*, for both forms of crystals were found commonly in cells whose color reactions indicated an acidity of at least $\text{pH } 1.5$. Judging by their color reactions, the specialized cells of *B. heracleifolia*, which ordinarily contained the tetragonal bipyramids, were no less acid than those of *B. corallina lucerna* which contained the monoclinic forms. In *B. ricinifolia* there was frequently more than one crystal in a single cell and sometimes both the tetragonal and the monoclinic forms appeared to occur in the same cell. It is possible, however, that the rosette aggregates were imperfectly developed crystals grown together in a stellate group which, as mentioned by DE BARY (2, p. 138) may appear to belong as well to one system as to the other.

The specialized crystal-containing cells were found among the parenchyma cells of the leaves of *Begonia* as well as in the stems and petioles. They were conspicuous in cross sections of even the youngest unfolded leaves around the apical bud. They occurred also in the flower stem and the branches of the inflorescence. They were not seen in the petals nor stamens, nor in the roots. In old stems and petioles of *B. lucerna* and *B. rex* occasional cells with much thickened and apparently pitted walls were observed. These cells took on colors corresponding to the highly acid forms of the indicators but did not contain crystals.

³ We are indebted to Mr. GEO. L. KEENAN, of the Food, Drug and Insecticide Administration for the identification of these crystals.

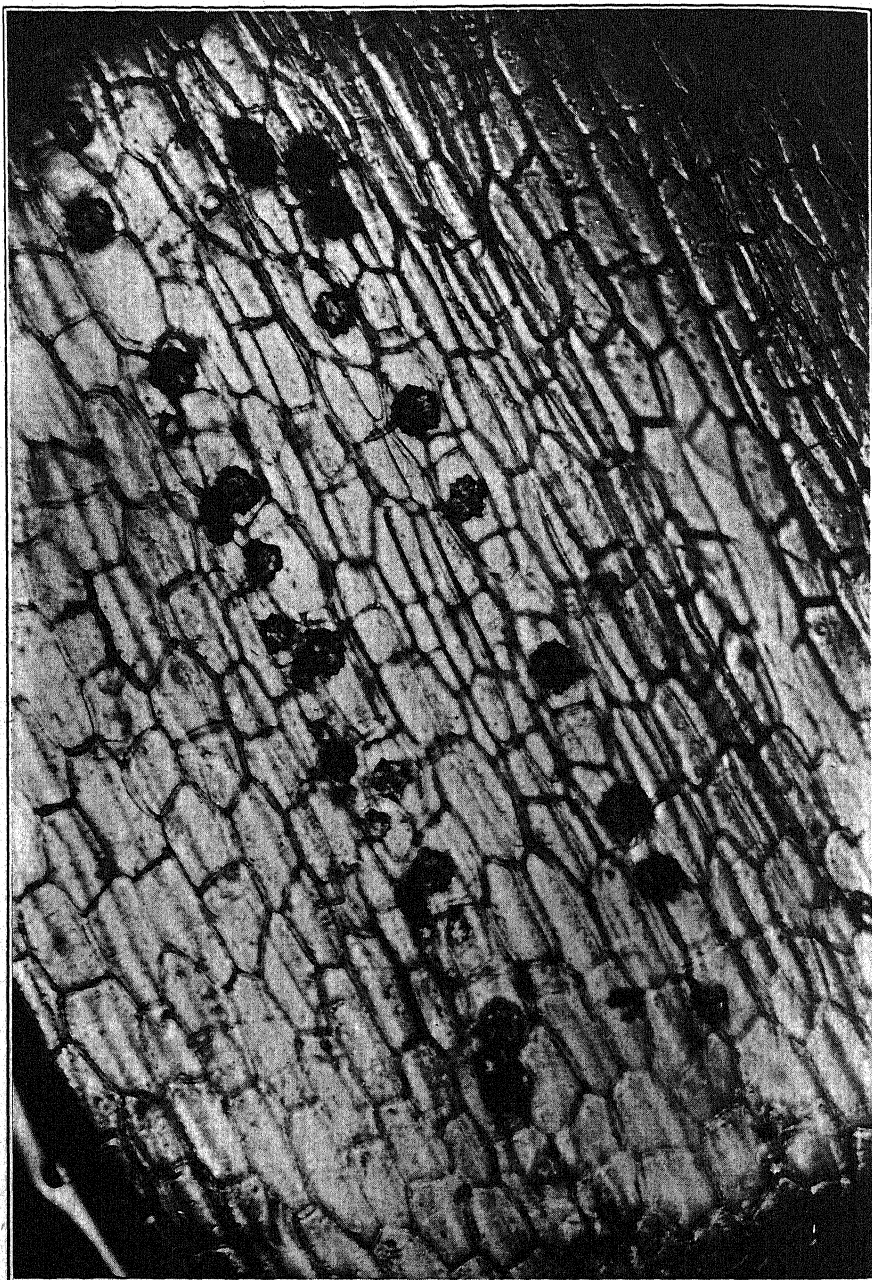


FIG. 3. Unstained epidermal tissue stripped from a petiole of *Begonia* sp. showing distribution of specialized cells containing crystals of calcium oxalate.

It became apparent early in the present investigation that because of the wide difference in the color of the ordinary cells under different conditions of staining, their acidity could not be satisfactorily determined by the usual method of staining with indicators. Thus, in solutions of brom cresol green, their color varied from yellow ($< \text{pH } 3.0$) to blue ($> \text{pH } 5.0$) according to certain external conditions. As previously described, these cells, which were first dyed yellow in brom cresol green, turned green within 15 minutes if the external yellow indicator solution were replaced with more of the original dye as fast as it became acidified by the acid leaching from the sections. If, however, the external solution were not renewed, they remained yellow for hours. Eventually, however, all but the specialized cells became green or blue while still immersed in the yellow indicator solution. The blue color usually appeared only after the section had lain for an hour or more in a relatively small quantity of a dilute solution (0.1 per cent.). When the section was more deeply immersed in a larger quantity of the dye, the blue color appeared much more slowly, if at all.

In itself, the appearance after about 15 minutes of this deep green or blue color would appear to indicate a reaction near $\text{pH } 5.0$ in the ordinary cells. However, there are several reasons for believing that this value does not represent the true acidity of the uninjured tissue. In the first place, there is such rapid diffusion of acid from the sections into the external dye solution that it would be unsafe to conclude that the reaction indicated by the colors appearing in the tissue after any appreciable interval of time is accurately indicative of the natural reaction of the cells. In the second place, the sections become yellow when first immersed in brom cresol green and turn green or blue only after some time has elapsed. In the third place, sections thinly covered with the indicator solution on a microscope slide often become blue, while those submerged more deeply in some of the same solution in a depression of the drop-plate remain yellow. This fact suggests that the availability of air has something to do with the appearance of the blue color. In the fourth place, the possibility that the green or blue color merely indicates the disappearance of the original acidity is also suggested by the fact that the acid which leaches from the tissue into the external indicator solution, and turns it from green or blue to yellow, disappears after a few hours, as shown by the gradual return of the original color of the solution. This change occurs only when the solution remains open to the air and in contact with the tissue.

It was found that the acidity of water in which pieces of tissue of *Begonia heracleifolia* had been left overnight varied greatly according to the depth of submergence of the material. When stems and petioles were cut up into small pieces and placed in a test tube, covered with distilled

water, and allowed to stand over night, the water became very acid by morning. When, however, similar tissue was barely submerged in the same volume of water in a watch-glass, the water became very acid at first but by morning was neutral to litmus. This experiment was repeated with *B. ricinifolia*, a few drops of brom cresol green being added to each preparation. The water in both the test tube and the watch-glass quickly became yellow as acid leached into it from the tissue. The next morning, the water in the test tube was still yellow ($< \text{pH } 3.0$), while that in the watch-glass had become a deep blue ($> \text{pH } 5.0$).

The only apparent difference between the two preparations was in the degree of exposure of the material to air. Accordingly, another experiment was set up, with *B. ricinifolia* petioles, to determine the effect of pulling air through the water by means of a suction pump. Two similar lots of tissue (2 grams each) were completely immersed in distilled water (5 cc.) to which a few drops of brom cresol green had been added. Air was bubbled vigorously through one preparation, while the other stood corked beside it. In less than an hour, the one through which air was bubbling was green, and in about an hour and a half it had become a deep blue. The other remained yellow. The experiment was repeated with similar results with *B. corallina lucerna* and with *B. lucerna*. It was found, also, that in no case did the acid disappear unless the solution was in contact with pieces of tissue.

This disappearance of the acid which leaches from the tissue indicates that the green and blue colors appearing in cells of *Begonia* sections immersed in brom cresol green, and the corresponding colors obtained with other indicators, result from oxidations or other reactions which take place within the cells when the tissues are exposed to air.

In order, therefore, to determine with greater certainty the reaction of the fresh tissues immediately after sectioning, single cells of the young stems and of petioles were punctured under the microscope in the presence of small quantities of various indicator solutions. A sharpened dissecting needle, bent at right angles about one-fourth inch from the point, was used to puncture the cells.

A vivid coloration corresponding to the highly acid form of the dye invariably appeared at the point of puncture regardless of whether the punctured cell was a specialized cell or an ordinary cell. The colors observed and the corresponding pH values are tabulated below.

Thus it was found that when the cells of freshly cut sections immersed in an indicator solution were punctured, there was no appreciable difference between the color appearing in the specialized cells and that in the ordinary cells. After about 15 minutes, *i.e.*, the time ordinarily taken for the highly

TABLE I

APPROXIMATE pH VALUES OBTAINED BY PUNCTURING SINGLE CELLS OF FRESHLY CUT TISSUES
OF *Begonia heracleifolia* IN INDICATOR SOLUTIONS

INDICATOR	COLOR IN PUNCTURED CELLS	pH VALUES
Methyl orange	pink	< 3.0
Brom cresol green	yellow	< 3.0
Brom phenol blue	yellow	< 2.5
Crystal violet	blue	1.5-1.7
Thymol blue	red	1.2-1.5

acid reaction of the ordinary cells to disappear when the sections were immersed in an indicator solution, puncturing the ordinary cells no longer resulted in the appearance of the highly acid form of the indicator. It appears that in freshly cut sections the ordinary cells are as acid as the specialized cells, while in older sections they are not. The two types of cells are evidently very different physiologically, inasmuch as the ordinary cells quickly lose their acid, while the specialized cells seem to retain it indefinitely.

Recently SMALL (24) has suggested a procedure for staining individual cells which consists in allowing cut sections of the plant to remain over night in a watch-glass containing dilute indicator solution. The colors in the cells as seen under the microscope the next morning are considered indicative of the cell acidity. Our investigation has shown that the error which results from the use of this method with such tissues as those of *Begonia* is very great. The difference between the apparent acidity of the cells of freshly cut sections of *Begonia* and those of sections which have stood over night in a dilute solution of brom cresol green amounts to several pH units. Thus all the cells of sections placed in the indicator in a watch-glass are yellow (< pH 3.0) for at least ten minutes after immersion, and show no color but yellow when punctured in the presence of the dye; but after standing over night in the dye, most of them (all except the specialized cells) are green or blue (> pH 5.0). If not submerged too deeply in the solution, this color change, from yellow through various shades of green to blue, may take place within two hours and sometimes less. Therefore, in order to accept the pH values indicated by the colors in cells which have stood in indicator solutions for some time, it is necessary to assume (1) that no significant quantity of acid leaches out from the cells during the interval, and (2) that the acidity has not been changed appreciably by chemical reactions taking place in the cells. There is considerable evidence that both these processes take place in *Begonia*.

Likewise the method suggested by SMALL (24) does not give an accurate indication of the relative hydrogen-ion concentrations in adjacent cells of *Begonia*. Thus, at first all the cells are the same shade of yellow in brom cresol green, the first differentiation being merely an intensification of the yellow in the crystal-containing cells. After an hour's immersion in the indicator these specialized cells become a reddish orange. During this time the background cells are becoming less and less yellow, turning to green under certain conditions and finally to blue. In brom phenol blue and thymol blue, equally striking differentiations are obtained. After several hours, the apparent pH values of the two types of cells may differ from each other by as much as two whole pH units. Yet, on puncturing them in the presence of indicators immediately after cutting, no evidence of any appreciable difference in their acidity can be obtained.

It is believed by some that oxidases are universally distributed in living plants (18). In view of the extremely high hydrogen-ion concentrations found throughout the tissues of *Begonia*, it was of interest to determine whether the usual oxidase tests could be obtained. Sections of stems, leaves and petioles were placed in solutions of benzidine, and of gum guaiacum. In both reagents the specialized cells became blue with the precipitate which is commonly assumed to indicate oxidase activity. Only traces of color appeared in the ordinary, unspecialized cells. The difference between the reaction in the two types of cells was especially striking in longitudinal sections in which the deeply stained specialized cells appeared in rows. Judging by their colors in indicator solutions, all these cells have hydrogen-ion concentrations of at least pH 1.5, at which reaction oxidases have been found to be inactivated (3, 19).

This paradoxical situation seems inexplicable except on the supposition that in spite of the appearance of uniform coloration in the cells, the protoplasm is isolated from the highly acid sap by an impermeable surface. Unlike the situation described by REED (17) for citrus fruits, the oxidase reactions in *Begonia* appear to be coincident with the high acid reactions. However, no reaction with either benzidine or gum guaiacum was obtained in the expressed juice; so it seems that in spite of lack of visible evidence of their separation, the enzymes and cytoplasm of *Begonia* are not in contact with the acid sap in uninjured cells. It is interesting in this connection to note that TUNMANN (27, p. 441), LUNDEGARDH (11, p. 202), and SCHAEDE (23, p. 219), found that various plant cells show an alkaline reaction in the cytoplasm and an acid reaction in the sap of the vacuoles. HAAS (9) expresses the opinion that in the highly acid cells of the cranberry, the acid sap is probably confined to vacuoles rather than imbibed in the protoplasm. REED (18) considers this to be generally true in highly acid

tissues. If this be the case in *Begonia*, the protoplasm must be limited to such a thin layer that its color reactions in indicators are masked by those of the acid sap occupying most of the space within the cell.

Summary

1. The freshly cut tissues of leaves, petioles and young stems of *Begonia* are characterized by hydrogen-ion concentrations corresponding to at least pH 1.5, which is in close agreement with electrometric determinations of the acidity of the expressed juice.

2. The usual method of vital staining by immersion of cut sections in solutions of indicators is not reliable for tissues such as those of *Begonia*, for the reason apparently that chemical changes which result in a decrease in the cell acidity occur in most of the cells when the sections are exposed to air. Thus the color of sections in brom cresol green changes from yellow (<pH 3.0) to green (pH 4.5) in 15 minutes, and to blue (> pH 5.0) in an hour, unless the sections are deeply immersed in the solution.

3. Although all the cells appear to be alike in having an extremely high acidity when the sections are first cut, nevertheless two physiologically different types of cells are sharply differentiated after the sections have been in the indicator solutions for about 15 minutes. Most of the cells rapidly lose their high acidity, as shown by a change in color toward the alkaline form of the dye, but others (the so-called "specialized" cells) seem to retain their acidity, the acid color of the dye persisting in them indefinitely. These specialized cells contain crystals of calcium oxalate and occur in rows lengthwise of the tissues.

4. Additional evidence of a physiological difference between the two types of cells appears when sections are immersed in solutions of the oxidase reagents benzidine and gum guaiacum. The specialized crystal-containing cells become blue in these reagents, while the rest of the tissue shows but a trace of color.

5. These oxidase reactions do not take place when the reagents are added to the expressed juice, apparently indicating that in uninjured cells the enzymes are not in contact with the acid sap.

BUREAU OF PLANT INDUSTRY,

UNITED STATES DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

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A MODIFIED KJELDAHL METHOD FOR THE DETERMINATION OF THE NITROGEN CONTENT OF YEAST

LEO M. CHRISTENSEN AND ELLIS I. FULMER

In previous studies on nitrogen fixation by yeast (2) it was noted that "according to the method of analysis used (Kjeldahl) there was a loss in nitrogen in the beginning and that actual gain in nitrogen was not apparent until after six or eight weeks. Moreover at the particular pH in which there was a maximum preliminary loss there was the maximum gain or fixation after the longer time interval." It was stated that "this phenomenon has been observed many times in our work and after considering all phases of the matter, our hypothesis is as follows: Yeast is known to be rich in ring nitrogen compounds and in the early stages of growth the nitrogen may be thrown into a compound not to be analyzed by the usual methods. Later in the growth these compounds may be transformed into materials amenable to the analysis used."

This communication deals with the development of a method by which larger percentages of nitrogen are obtained from yeast than are secured by the usual Kjeldahl procedure. Many modifications of the Kjeldahl method have been described by HEPBURN (4). The use of hydrogen peroxide during digestion has been proposed by BERMAN (1), HEUSS (5) and KLEEMAN (6).

The three general methods for nitrogen determination in organic compounds are:

1. Dry combustion and oxidation to elementary nitrogen (Dumas).
2. Liberation of the nitrogen as NH_3 by heating with an alkali.
3. Wet combustion and reduction of all nitrogen to ammonia (Kjeldahl).

The Dumas method is perhaps the most universally applicable although it tends to give high results. Preliminary experiments with the first two methods with dry and fresh yeast gave the results shown in Table I as an average of four determinations.

From these results it is apparent that some other method of analysis than the usual Kjeldahl is essential in order to follow the change in nitrogen content of a medium in which yeast is growing. The Dumas method is not applicable because of the time required for a determination and because of the difficulty in handling large amounts of material with so little nitrogen. Moreover, the method is not suitable for use with the volumes of liquid used

TABLE I
PERCENTAGE OF NITROGEN

YEAST	DUMAS (a)	KJELDAHL (b)	$\frac{b}{a} \times 100$
Dried	9.00	7.95	88.5
Fresh	2.24	2.02	90.1

in biological work. The alkali distillation methods seem never to have been very generally applicable and only one, the TER MEULEN method, was tried.

Ter Meulen method

H. TER MEULEN (8, 9) devised a modification of the old soda lime process with some real improvements. The sample is well mixed with anhydrous sodium carbonate and a little reduced nickel and placed in a quartz combustion tube. The rest of the tube is filled with asbestos coated with reduced nickel. Hydrogen, freed from ammonia and saturated with water vapor, is passed over the sample and then over the reduced nickel, both at 350° C. The gases issuing from the combustion tube are passed through a condenser and through a dilute acid. Ammonia may be determined in any of several ways. He obtained very good results with this method in the analysis of several kinds of flour, coal, oil cake, casein, gelatin, glue and other materials, and stated that the analysis required only a few minutes. In general higher results are obtained than by the Kjeldahl method. This seemed to offer possibilities in the analysis of yeast but even with several hours of heating and with temperatures of 800–900° C., the best result obtained was 6.05 per cent. nitrogen, which is but 75 per cent. of the Dumas result and considerably lower than the Kjeldahl. Obviously this method is not suited to the analysis of nitrogen in yeast.

Some modification of the Kjeldahl method would seem most desirable. The following experiments were carried out in an attempt to devise a more suitable Kjeldahl method.

Variation in catalyst

A large number of modifications is based on the use of some particular catalyst. The Gunning modification made use of potassium sulphate, which acts by raising the temperature of the digestion and therefore is not strictly a catalyst, and this more than any other factor affects the rate of digestion. Without K_2SO_4 (H_2SO_4 alone) it required 6 hours to digest 0.5 gm. of

yeast, while with potassium sulphate present the digestion was shortened to 2 hours and 30 minutes. The maximum amount of potassium sulphate that can be used without solidification of the digestion mass on cooling is 10 gm. per 25 cc. of sulphuric acid.

Many metals or their salts have been used as catalysts, the most common being copper or mercury. Other metals tried were vanadium, chromium, and manganese or their salts but the results showed that these possessed no advantage over the copper or mercury.

It was found in all cases advisable to continue the digestion for three hours after the mixture became clear in order to obtain maximum yields of nitrogen from the yeast; more or less than this heating tended to give lower results.

Addition of oxidizing agents to the sulphuric acid during or at the end of digestion

A large number of modifications is based on the use of oxidizing agents during or at the end of digestion. KMnO_4 is commonly used. Directions generally are to add one or two crystals at the end of the digestion, and to avoid an excess, as indicated by a blue or purple color. Persulphates and hydrogen peroxide have been employed by a number of investigators. They are generally added early in the digestion.

It was found that potassium permanganate does not give higher yields of nitrogen from yeast when added in varying amounts either during or at the end of the digestion; but if added in excessive amounts (sufficient to cause purple color) there is a decided lowering of the nitrogen obtained, especially if the solution is heated after the addition of KMnO_4 .

H_2O_2 in the form of a 30 per cent. solution known as perhydrol or superoxol was added during digestion, in quantities of 5 to 15 cc. Although the digestion was materially hastened by the addition of such amounts, there was no increase in the yield of nitrogen, although like KMnO_4 it was apt to cause lowered yields when added in too large quantities. When it is used, a blank must always be run to take care of the nitrogen in the superoxol, which may amount to 15 mg. per 100 cc. Several other oxidizing agents were used. V_2O_5 , CrO_3 , MnO_2 did not appear to have any beneficial effect but were apt to be detrimental when added in large amounts.

Preliminary hydrolysis and oxidation

The hypothesis was advanced that certain ring nitrogen compounds in the yeast did not yield all their nitrogen in the usual Kjeldahl method. If this is true it should be possible to render these compounds more amenable to analysis by an acid hydrolysis and oxidation preliminary to the digestion with concentrated sulphuric acid.

Refluxing 0.5 gm. of yeast with 30 cc. of water to which was added 1 cc. of concentrated sulphuric acid and then digesting in the usual way give slightly higher results than were obtained by the usual Kjeldahl procedure. Thus yeast which yielded 7.80 per cent. nitrogen by the Kjeldahl method gave 7.90 per cent. by this method. But when superoxol was added in this acid hydrolysis there was a very marked improvement. The preliminary experiments were carried out with 0.5 gm. of yeast which was placed in a 500 cc. Kjeldahl flask and treated with 30 cc. of 20 per cent. H_2O_2 in water, 1 cc. of concentrated sulphuric acid, 10 gm. of K_2SO_4 and 0.4 gm. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and heated over a low flame in the digestion rack. When the contents of the flask were reduced almost to dryness the flame was removed and the flask allowed to cool and 24 cc. of concentrated sulphuric acid were added and the digestion carried out in the usual way. The amount of hydrogen peroxide and of sulphuric acid were found to be important. The optimal results were obtained by the use of 15 per cent. H_2O_2 by weight,¹ in the mixture to which were added the potassium sulphate, copper sulphate and sulphuric acid. With more than 1 cc. of sulphuric acid lower results were obtained while less acid gave erratic results. This method gave results considerably higher than the Kjeldahl method, yielding about 95 per cent. of the nitrogen obtained by the Dumas method.

It is apparent that the method involving the preliminary acid hydrolysis and oxidation with 15 per cent. H_2O_2 gives results with yeast more nearly approaching those obtained by the Dumas method. This method was then compared with the Dumas and the ordinary Kjeldahl for several organic compounds and organisms. The yeast nucleic acid and nucleo-protein were prepared by the method described by Hawk (3). The results of such analyses are given in table II.

The modified Kjeldahl method gave results on yeast nucleic acid and yeast nucleoprotein as well as on dried and fresh yeast approaching those obtained by the Dumas method, and considerably higher than those given by the Kjeldahl method. When Fleischmann's yeast was dried in a vacuum at 40° C. there was practically no difference in the nitrogen content as determined by the three methods, whereas there was a great difference when the same yeast was dried at atmospheric pressure and room temperature. Dried yeast seemed to lose nitrogen on storage, as indicated by the Dumas method, but there was a much lower loss as measured by the Kjeldahl method. These phenomena would indicate that some of the compounds not amenable to the Kjeldahl method are volatile.

The heterocyclic ring compounds analyzed gave very nearly the same results by all three methods. It will be noted that diphenylamine gave

¹ The amount of H_2O_2 present was determined by the permanganate method according to Merck (7).

TABLE II

NITROGEN ANALYSIS OF SEVERAL SUBSTANCES BY THREE METHODS

COMPOUND OR MATERIAL	PERCENT OF NITROGEN FOUND				$\frac{b}{a} \times 100$	$\frac{c}{a} \times 100$
	CALCULATED	DUMAS (a)	KJELDAHL (b)	MODIFIED KJELDAHL (c)		
Caffeine	28.9	29.77	28.63	27.04	96.2	90.8
Uric acid	33.3	33.24	31.36	31.58	94.3	95.0
Theobromine	31.1	31.79	30.56	29.62	96.1	93.2
Diphenyl amine	8.3	8.62	8.18	4.40	94.9	51.4
Casein		14.89	13.66	13.88	91.7	93.20
Penicillium expansum, powdered, air dried. Mycelium and spores		5.23	4.66	5.00	89.1	95.6
Aspergillus niger, powdered, air dried. Mycelium and spores.....		4.49	4.42	3.98*	98.5	88.9
Yeast nucleic acid.....		7.78	6.72	7.58	86.4	97.4
Yeast nucleoprotein.....		14.85	12.14	13.59	81.7	91.5
Yeast, Fleischmann, dried at factory.....		9.00	7.95	8.60	88.3	95.6
Same yeast one year later		8.28	7.88	7.93	95.2	95.8
Yeast, Fleischmann, fresh, air dried.....		8.46	7.17	7.45	84.7	88.0
Yeast, Fleischmann, fresh, vacuum dried..		7.54	7.58	7.63	100.5	101.2
Yeast, Fleischmann, fresh		2.24	2.01	2.28	89.7	102.

* These are the highest results obtained by several determinations.

very much lower results by the modified method, due to volatilization during the preliminary treatment. Of the two molds analyzed, one gave a higher result by the modified method and the other a very much lower result. Casein contained more nitrogen as determined by the modified than by the regular Kjeldahl method.

The results indicate that for maximum yields of nitrogen the method needs to be adapted to the particular material to be analyzed. The method developed in this work is well suited for the analysis of yeast and yeast nucleo-protein and nucleic acid.

Summary

The following modified Kjeldahl method is proposed for the analysis of nitrogen in yeast. The sample is suspended in 20–30 cc. of a solution con-

taining 15 per cent. hydrogen peroxide by weight to which are added 10 grams of potassium sulphate, 0.4 gram of copper sulphate crystals and 1 cc. of concentrated sulphuric acid. The mixture is evaporated almost to dryness over a low flame. After the residue is cool it is digested according to the regular Kjeldahl method. This method gives 96-100 per cent. of the nitrogen in yeast according to the Dumas method. This method is not necessarily advantageous for all materials but the optimum concentration of peroxide may need to be determined in each case.

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THE INFLUENCE OF DIRECT IRRADIATION BY A QUARTZ MERCURY ARC LAMP UPON THE GERMINATION AND GROWTH OF CERTAIN SEEDS

CHARLES SHEARD AND GEORGE M. HIGGINS

(WITH FIVE FIGURES)

In a previous paper (4) we submitted certain data concerning the effects produced on the germination of seeds and the growth of seedlings under restricted periods of selective irradiation as obtained by the use of filters which screened out, by successive steps, the various portions of the ultra-violet light radiated by a quartz mercury arc lamp. In this article there are presented determinations on the germination of common garden seeds and the growth of seedlings when irradiated for one, two, five and ten minutes every twenty-four hours respectively under the same lamp and when kept in darkness or under the subdued daylight as transmitted into the room by ordinary window glass.

Experimental conditions

An air-cooled quartz mercury arc lamp* was used and operated at 70 volts and at a distance of 50 cm. The lamp was standardized and found to give a grade 1 reaction, or transient erythema, of the normally unexposed skin of the upper arm in three minutes at a distance of 50 cm. and a grade 2 effect, or permanent erythema, in six minutes.

Carefully selected seeds of lettuce, radish and turnip were placed upon moist blotters in suitable and similar glass containers, and each was covered with a piece of ordinary window glass of the same thickness which was removed only at the time of irradiation. Conditions were maintained as uniformly as possible with respect to temperature, moisture and methods of handling the seeds and seedlings. The seeds contained in two of the jars were kept as normal controls; those contained in the remaining jars were irradiated from above for one, two, five and ten minutes, respectively, two jars and contents being used for each period of exposure. One jar of each pair was then placed in darkness until the next period of irradiation; the second one in each case was subjected to the full complement of diffuse daylight present in the room and was exposed, therefore, to such infra-red, visible and ultraviolet radiations as penetrate window glass. Observations

* The lamp used during these observations was made available through the courtesy of the Victor X-Ray Corporation.

were made at the end of the first twenty-eight hour period and at each twenty-four hour period thereafter. Measurements (millimeters) were taken on the growth of the seedlings attained at the end of each period. In order to differentiate the terms germination and growth, we are using here, as in the previous paper, the word germination to include the earlier and inner growth prior to the first external appearance of the seedling root or radicle.

Experimental results

Tabulations of the experimental data obtained under various periods of irradiation and subsequent disposition of the seedlings (that is, whether kept in the subdued daylight of the room or in a dark cabinet) are given in tables I, II and III. In figures 1 and 3 are shown graphically the effects of irradiation by a quartz mercury lamp on the growth of seedlings of lettuce and turnip, respectively, when kept in darkness; figures 2 and 4 show the effects of such irradiation upon like seedlings when kept under the indirect and subdued daylight of the room.

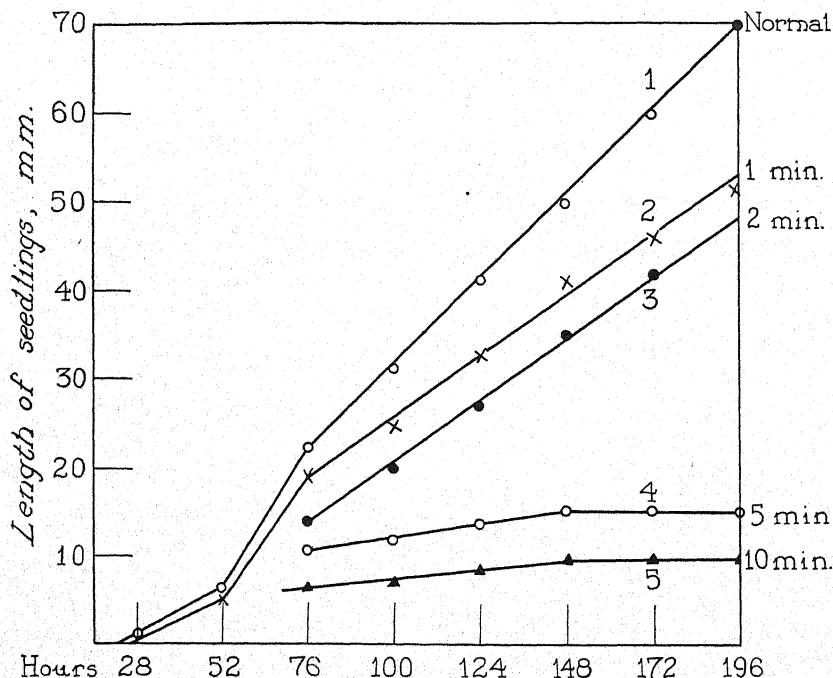


FIG. 1. Relationships between the lengths of seedlings (mm.), daily period of irradiation (minutes) and number of periods of irradiation in the case of lettuce seed grown in darkness. Curve 1, normal control; curve 2, one minute irradiation at 50 cm. by a quartz mercury arc operated at 70 volts; curve 3, two minutes daily irradiation; curve 4, five minutes daily irradiation; curve 5, ten minutes daily irradiation.

TABLE I

DAILY GROWTH OF LETTUCE SEEDLINGS (MM.) UNDER SPECIFIED EXPERIMENTAL CONDITIONS

TIME ELAPSED	IRRADIATED								NON-IRRADI- ATED CON- TROLS	
	1 MINUTE DAILY		2 MINUTES DAILY		5 MINUTES DAILY		10 MINUTES DAILY			
	Hours	Dark*	Light**	Dark	Light	Dark	Light	Dark	Light	Dark
28	1	0.5	2	1	2.0	2	2.0	2	3	0.0
52	5	2.5	3	4	6.0	5	0.6	5	7	0.5
76	18	6.0	14	12	11.0	15	7.0	12	25	1.0
100	24	11.0	20	19	12.0	15	7.5	12	33	1.0
124	35	20.0	27	25	13.5	14	9.0	12	42	4.0
148	42	30.0	35	35	15.0	13	10.0	13	50	4.0
172	46	35.0	42	40	15.0	13	10.0	13	60	12.0
196	50	40.0	47	52	15.0	13	10.0	13	70	12.0

All seeds and seedlings irradiated by a mercury-quartz lamp, operated at 70 volts, at a distance of 50 cm.

* Grown in a dark cabinet.

** Grown under subdued or interior daylight as transmitted by ordinary window glass.

EFFECTS OF IRRADIATION ON GERMINATION

SEEDLINGS KEPT IN DARKNESS.—Different effects appear to be produced on the germination of seeds when they are irradiated by the quartz mercury lamp, depending on whether the seeds are subsequently kept in the dark or under diffuse daylight. From the data in the tables it will be seen that germination is most rapid in normal, non-irradiated seeds kept in darkness and least rapid for similar normal, non-irradiated seeds kept under interior daylight. Irradiation by the mercury lamp does not appear to aid in the germination of seeds kept in darkness but apparently retards it slightly.

SEEDLINGS KEPT UNDER DIFFUSE DAYLIGHT.—The converse of the last statement of the foregoing paragraph, however, is true in the case of seeds kept in subdued daylight. We may conclude, therefore, that the germination of seeds, which normally germinate underground and in darkness, is inhibited by interior daylight, which is possessed of practically no radiation of wavelength less than 370 m μ , hence consisting of visible and some infra-red radiations only. Apparently ultra-violet radiation does not accelerate the endogenous growth of the seeds when kept under physiologic or normal conditions; such radiation, however, definitely stimulates germination in seeds which are in an unphysiologic environment. An initial irradiation of ten minutes apparently produces the same rate of germination in seeds,

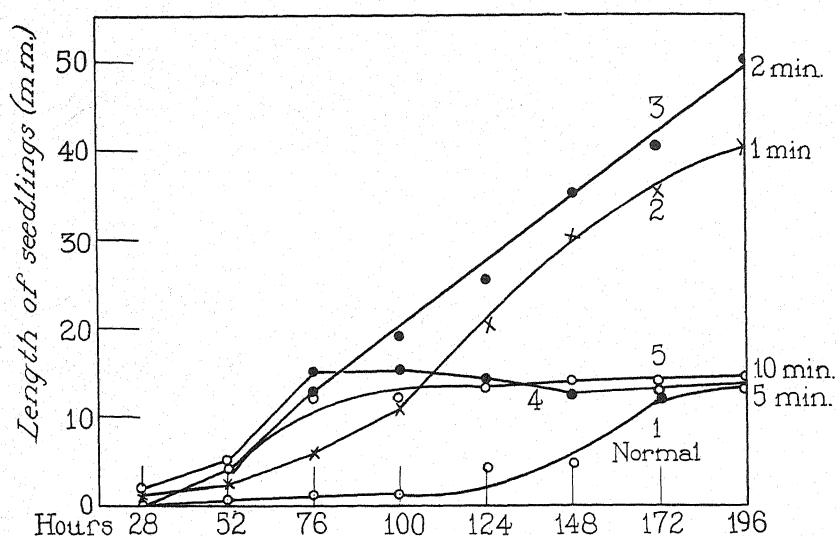


FIG. 2. Relationships between the lengths of seedlings (mm.), daily period of irradiation (minutes) and number of periods of irradiation in the case of lettuce seeds grown under *subdued daylight*. Curve 1, normal control; curve 2, one minute daily irradiation at 50 cm. by a quartz mercury arc operated at 70 volts; curve 3, two minutes daily irradiation; curve 4, five minutes daily irradiation; curve 5, ten minutes daily irradiation.

TABLE II

DAILY GROWTH OF RADISH SEEDLINGS (MM.) UNDER SPECIFIED EXPERIMENTAL CONDITIONS

TIME ELAPSED	IRRADIATED								NON-IRRADIATED CONTROLS	
	1 MINUTE DAILY		2 MINUTES DAILY		5 MINUTES DAILY		10 MINUTES DAILY		Dark	
	Dark	Light	Dark	Light	Dark	Light	Dark	Light		
28	2	0	2	0	2	1	2	1	4	0
52	10	0	8	4	7	6	6	5	12	0
76	28	2	22	12	14	25	18	25	38	0
100	40	5	32	30	15	25	18	25	50	0.5
124	55	18	45	45	17	22	19	22	75	2.0
148	70	30	60	50	18	20	20	20	105	3.0
172	87	40	68	60	19	20	20	19	120	15
196	108	55	75	60	19	20	20	19	145	16

whose growth and germination are inhibited by the longer wavelengths of daylight, as occurs in normal, non-irradiated seeds kept in darkness.

EFFECTS OF IRRADIATION ON GROWTH

SEEDLINGS KEPT IN DARKNESS.—Maximal growth was attained by the normal, non-irradiated seedlings kept constantly in darkness, as is evidenced

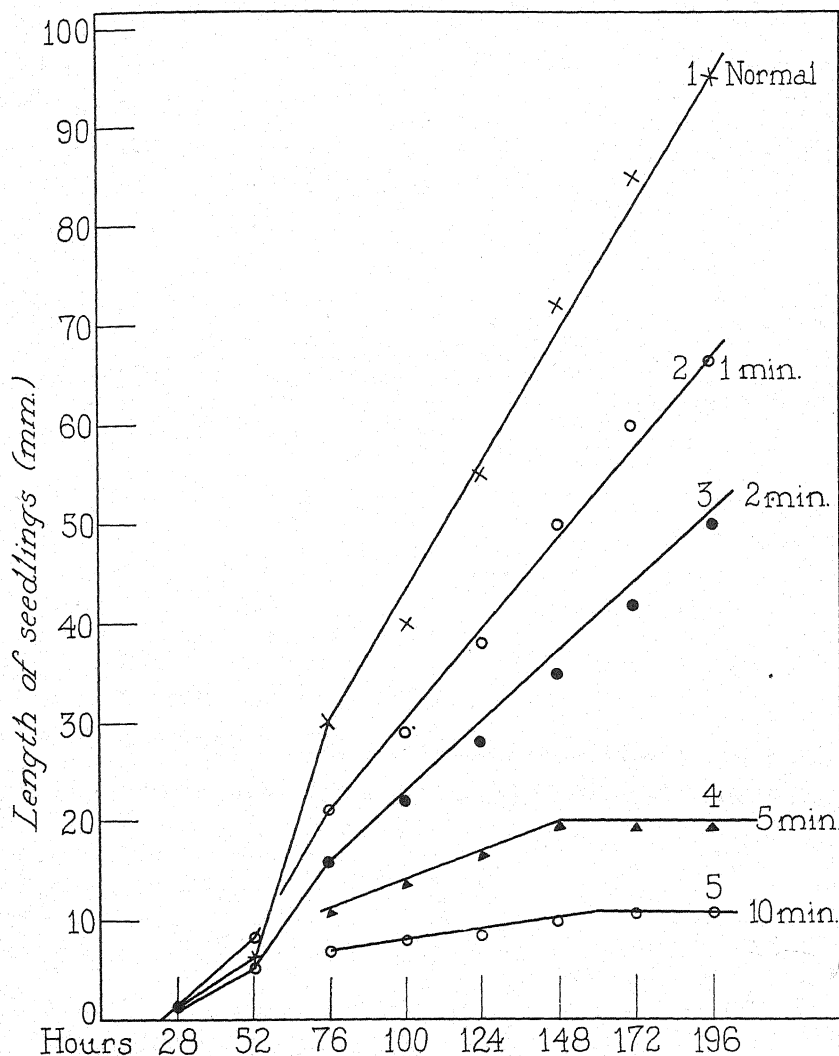


FIG. 3. Relationships between the lengths of seedlings (mm.), daily period of irradiation (minutes) and number of periods of irradiation in the case of turnip seeds grown in darkness. Curve 1, normal control; curve 2, one minute irradiation daily; curve 3, two minutes daily irradiation; curve 4, five minutes daily irradiation; curve 5, ten minutes daily irradiation.

in curve 1 of figure 1 and figure 3. The rate of growth is materially lessened as the length of the period of irradiation is increased when the seedlings are reared in darkness. The curves also show that seedlings which have been irradiated for five or ten minutes each day practically cease

TABLE III

DAILY GROWTH OF TURNIP SEEDLINGS (MM.) UNDER SPECIFIED EXPERIMENTAL CONDITIONS

TIME ELAPSED	IRRADIATED								NON-IRRADIATED CONTROLS	
	1 MINUTE DAILY		2 MINUTES DAILY		5 MINUTES DAILY		10 MINUTES DAILY			
	Hours	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
28	2	0	2	0	2	1	2.0	1	2	0.0
52	8	1	6	2	8	4	6.0	4	6	0.5
76	21	8	16	8	11	17	7.0	12	30	0.5
100	29	18	22	20	14	17	8.0	12	40	1.0
124	38	25	28	25	17	18	8.5	12	55	4.0
148	50	30	35	35	20	18	10.0	11	75	4.0
172	60	35	42	40	20	18	11.0	11	85	5.0
196	66	40	50	45	20	18	11.0	11	95	0.5

growing after five days. We may infer, therefore, that such quantities of irradiation from the quartz mercury lamp are lethal. In fact it can be shown from considerations which will be presented in a subsequent communication that the lethal effects of irradiation on seedlings raised in darkness are cumulative and that, under the conditions of our experiments, a total period of irradiation of about one hundred minutes would prevent any growth of seedlings. The natural habit of such seedling roots is underground, where they receive little if any light and hence no ultra-violet radiation. Ultra-violet radiation is inhibitory in its action and lessens the rate of growth of the rootlets, probably because of changes which, if carried to their extreme, eventuate in coagulation of the seed contents. Such, however, is not true in the case of the growth of the stems of lettuce, turnip and radish plants, for we have found that the maximal growth occurs under selective filters which permit of the passage of the "near"-ultra-violet, violet and blue portions of solar energy.

SEEDLINGS KEPT UNDER DIFFUSE DAYLIGHT.—When seedlings are kept under conditions of indoor daylight we find considerably different results from those which have just been described. Minimal growth was attained by normal, non-irradiated seedlings kept under maximal periods of diffuse daylight as transmitted by ordinary window glass. In the light thus transmitted there is no appreciable ultra-violet content below a wavelength of 370 m μ ; hence, practically speaking, no ultra-violet portion.

The data in the tables and graphically shown in the curves of figures 2 and 4 show that optimal conditions for continuous maximal growth of the seedlings kept under interior daylight were attained under periods of irradiation of from two to three minutes a day. It is also evident that two

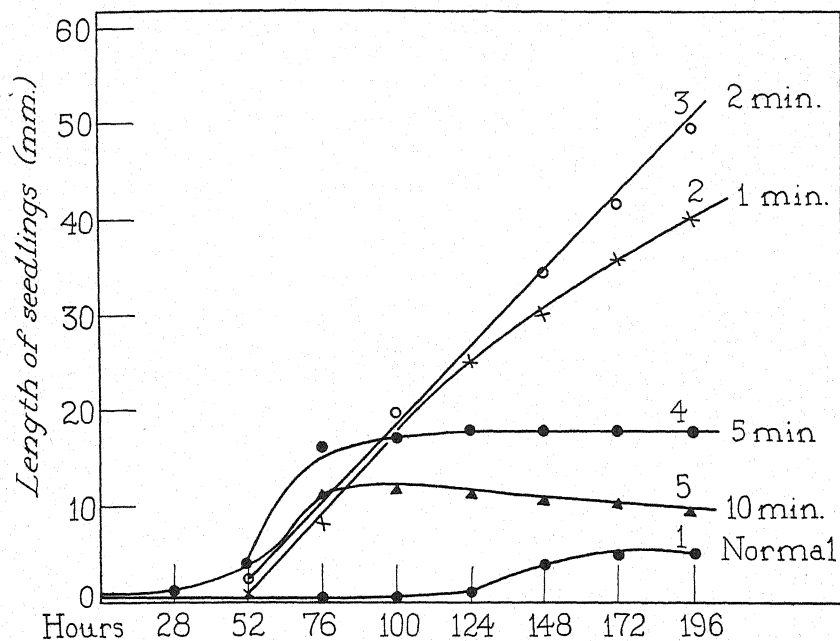


FIG. 4. Relationships between the lengths of seedlings (mm.), daily period of irradiation (minutes) and number of periods of irradiation in the case of turnip seeds grown in *subdued daylight*. Curve 1, normal control; curve 2, one minute daily irradiation; curve 3, two minutes daily irradiation; curve 4, five minutes daily irradiation; curve 5, ten minutes daily irradiation.

or three periods of daily irradiation, of from five to ten minutes each, induce the maximal rate of growth. Under such circumstances, however, the maximum of growth is quickly reached and passed, due without doubt to the fact that, coupled with the stimulative effects on the endogenous growth of the cells of the rootlet, there is also a lethal effect produced by reason of the fact that the exogenous metabolism of the cells is interfered with by virtue of changes in the permeability of cell membranes or cell contents. The immediate changes produced are of the same essential nature as those caused by temporary exposure to high temperatures or other activating agents. The degree of growth attained at the end of three or four periods of ultra-violet irradiation, of from five to ten minutes each, is maintained for several days, indicating that the factors making for growth are approximately balanced by those tending toward death and annihilation. We may, in passing, very properly raise the question whether the maximal growth is attained under irradiation for three daily periods of five to ten minutes each and whether growth is then suddenly terminated. It would

seem to be just as logical and as probable that the stimulative effects of the initial periods of irradiation are carried over into the growth of the few succeeding days and that the lethal effects of irradiation have obtained from the very beginning. Incidentally, the series of results reported here are similar in general to the effects we have found to be produced by ultra-violet radiation from a quartz mercury arc lamp on the early larval development of *Rana pipiens* (5).

In figure 5 we have plotted the lengths (mm.) of seedlings grown in darkness (in the case of the experiments on turnip seeds only) as ordinates and the total time of irradiation as abscissae. We have assumed implicitly that all the seedlings in the five groups would have reached the same length at the end of any given day (for example, at the end of seventy-six hours, a length of 30 mm. and marked as the point *a* on curve 1, figure 5) if all of them had been treated as normal, non-irradiated seeds and seedlings kept constantly in darkness. The point marked *c* on curve 1 represents a total growth of 21 mm. at the end of the same seventy-six hours under a total irradiation from the quartz mercury lamp of three minutes; point *e*, six minutes, and so on. The line *ab* may be taken to represent the normal growth of a non-irradiated seedling kept in darkness, since it is a line connecting points (lying on the axis representing length of seedlings) which show the daily growth from the third day on (for example, the point *a* at the end of seventy-six hours) and under zero daily period of irradiation. The line *cd* joins points which indicate the length of the seedling after it has been irradiated one minute daily on successive days; the line *ef* passes through points plotted to show the lengths attained under daily periods of irradiation of two minutes; and so on, for the lines *gh* and *ij*. The slopes of these lines indicate that the daily amount of retardation in growth and therefore the rates of retardation in growth vary with the length of the daily period of irradiation. If, therefore, we are desirous of ascertaining the rate of retardation or percentage change in the growth of the seedling for a daily period of irradiation of one minute as given by the mercury vapor quartz lamp, we simply need to subtract the values as given on the line *cd* from those shown for corresponding days on the line *ab*, thereby obtaining the amounts of retardation in growth (mm.). In the particular instance we have cited, we have the following data:

Length of seedling on line <i>ab</i> (mm.)	Length of seedling on line <i>cd</i> (mm.)	Retardation	
		(mm.)	Per cent.
30	21	9	30
41	29	12	28
55	38	17	31
75	50	25	33

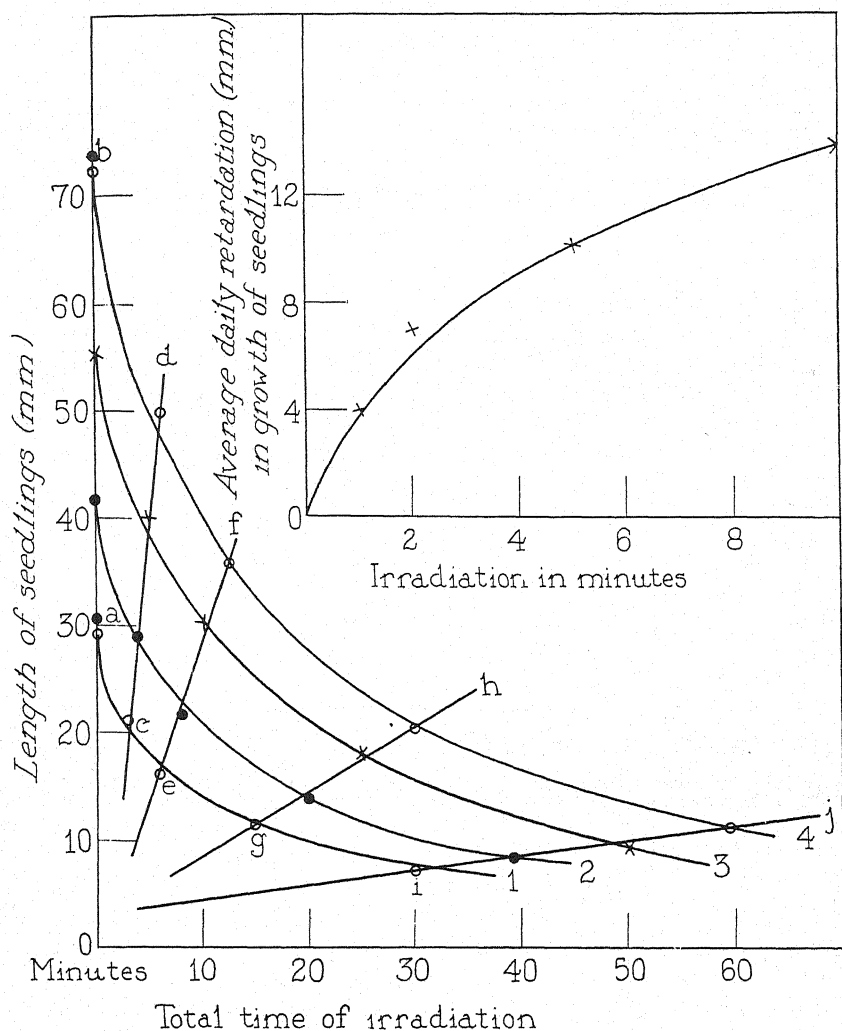


FIG. 5. Relationships between the lengths (mm.) of turnip seedlings grown in darkness and the total time of irradiation (minutes) by an air-cooled quartz mercury lamp. In curve 1, the point *a* represents the normal growth reached at the end of seventy-six hours; point *c*, growth attained under a total irradiation of three minutes (one minute daily); point *e*, total irradiation of six minutes; point *g*, total irradiation of fifteen minutes. The lines *cd*, *ef*, *gh* and *ij* indicate the rates of retardation (mm.) of growth under various daily periods of irradiation.

The inset in the upper right-hand corner shows the average daily retardation (mm.) of growth of seedlings (kept in darkness) for various daily periods of irradiation.

By simple calculation, therefore, we find that the daily retardation in growth and the time of irradiation are correlated as follows:

Length of daily period of irradiation (minutes)	Retardation (Per cent.)
1	30
2	50
5	70
10	80

The insert in the upper right-hand portion of figure 5 shows the average daily retardation (mm.) of growth of seedlings, kept in darkness, for various periods of irradiation. The curve is for turnip seedlings (table III). For example, the value of mm. daily retardation of growth under a daily irradiation of one minute by the mercury quartz lamp operated at 70 volts and at a distance of 50 cm. from the seeds is obtained by taking the differences between curves 1 and 2 of figure 3 at each successive twenty-four-hour period and dividing these differences by the number of periods of irradiation.

Discussion

We have not attempted to measure the energy* received by the seeds and seedlings under the conditions of our experiments. In fact, if one attempts to measure radiant energy, the problem is complicated, for there is no single instrument or method which will measure heat, light and ultra-violet rays with equal completeness and accuracy. However, in these experiments the same lamp was operated at the same voltage and distance in each instance;

* Since this paper was prepared we have measured the distribution of energy and the total radiation from the air-cooled mercury vapor lamp used in these experiments; when operated at various voltages. A Coblentz thermopile (12 junctions, iron and constantan) and a Coblentz galvanometer, both of which were properly mounted and screened, were employed for the measurement of the radiant energy. The standard source of radiation was a carbon filament incandescent lamp, calibrated by the Bureau of Standards. The intensity of radiation, as certified by the Bureau, is 52.3×10^{-8} watt for each square millimeter of receiving surface, when the lamp carries a current of 0.300 amperes under 96.6 volts and is placed at a distance of 2 meters from the receiving instrument. After obtaining the deflections of the galvanometer when the thermopile is exposed to the standard lamp (operated at a distance of 2 meters under the conditions previously specified) and the air-cooled quartz mercury vapor lamp (operated at 70 volts and at a distance of 3 meters), it is possible to calculate the watts for each square millimeter received by the thermopile when the mercury arc is at a distance of 50 cm. from the receiving instrument. Such a calculation shows that the energy received by the seeds and seedlings when exposed to the lamp operated at 70 volts and at a distance of 50 cm. is 5650×10^{-8} watt. With appropriate selective filters it was found that the distribution of energy from the lamp was approximately 30 per cent. infra-red, 33 per cent. visible, and 37 per cent. ultra-violet radiation.

hence the energy received may be safely assumed to be proportional to the time of irradiation.

In searching the literature (which we find sparse and difficult to obtain) we find some statements and conclusions which are in support, in whole or in part, of the findings in this paper. On the other hand, there are some apparent disagreements. The spores and seeds of certain plants (for example, the spores of some ferns and *Viscum* seeds) have long been said not to germinate in darkness. Other seeds seem to be delayed or prevented from germination by light (7), as in the case of the seeds we have investigated. By modifying the conditions, however, and by further consideration of the natural habitat for germination of the seeds concerned, germination may nevertheless take place. These results are, of course, of practical importance to nurserymen, florists and farmers. An excellent résumé of this subject is given by MOLISCH (8).

The observations of KLEBS (6) on the influence of light of different wave-lengths or colors (qualities) and amounts (quantities) of energy, making use of accurate means of quantitative determination, show that the germination of the spore of the fern (its cell division and cell growth) the growth of the plant (prothallus) in length, breadth and thickness, as well as the formation of the organs of sexual reproduction, are greatly affected by both the amounts and qualities of the radiant energy. Thus KLEBS showed that, under ordinary conditions, fern spores will not develop in darkness, neither does illumination with blue, violet or ultra-violet radiations stimulate them to growth. On the other hand, cell division and cell differentiation are said to be more strongly influenced by light rays of the lesser wavelengths (blue and violet) than of the greater wavelengths (red).

Many theories, couched in more or less technical terms, have been advanced to explain the stimulating effects of light on living protoplasm and living organisms (2). Critical reviews of these are to be found in the writings of BLAAUW and of KÖNIGSBERGER. But it is certainly not possible at the present time to reach an adequate explanation of how the living cell and the living organism are affected, chemically and physically, by light. Changes in the permeability of the cell membranes and in the nature and rate of the intracellular chemical reactions may be conjectured, but this is all very incomplete. Still, the influence of photochemical or other rays on the permeability of the cytoplasmic membranes must be considered as being involved in germination, growth, and movement (1, 3).

Conclusions

1. In seeds which normally germinate and grow in darkness and underground, the most rapid germination and maximal growth were attained by the normal, non-irradiated seeds and roots kept constantly in darkness.

2. The least rapid germination and minimal growth were attained by normal, nonirradiated seedlings kept under maximal periods of diffuse daylight as transmitted by ordinary window glass. In the light transmitted there is no appreciable ultra-violet content below 370m μ ; hence, practically speaking, no ultra-violet portion.

3. The action of diffuse daylight is to inhibit germination of seeds and growth of roots. Since there is a preponderance of greater wavelengths and an absence of ultra-violet radiations in subdued interior daylight, it is evident that the greater wavelengths inhibit (or at least do not stimulate) the germination of seeds and the growth of roots of those seeds which normally germinate and grow underground.

4. Irradiation by a quartz mercury lamp accelerates the germination of seedlings kept in subdued interior daylight as compared to the germination of normal non-irradiated seeds under similar conditions.

5. In general, optimal conditions for continuous maximal growth of seedlings kept in interior daylight are attained under irradiation periods of two to three minutes each day.

6. The stimulus to most rapid germination of seeds kept under interior diffuse daylight is an initial irradiation of from five to ten minutes. Longer periods of irradiation appear to have no additional stimulative effect. Two or three periods of daily irradiation of from five to ten minutes each, induce the maximal growth in seedlings kept under interior daylight. Therefore we may believe that such quantities of irradiation are able to counteract the untoward conditions relative to germination and growth induced by daylight.

7. These experiments, *in toto*, lend support to the hypothesis that ultra-violet radiation in the so-called biologic or "near"-ultra-violet region aids in the germination and growth of a cell or in the normal function of an organism which is kept in an unphysiologic environment.

8. These experiments also support the hypothesis that biologic or "near"-ultra-violet radiation stimulates the endogenous growth of the cells and of the organism as a whole, while the greater wavelengths of visible radiation influence the exogenous metabolic processes.

SECTION ON PHYSICS AND BIOPHYSICAL RESEARCH
AND DIVISION OF EXPERIMENTAL SURGERY AND PATHOLOGY,
MAYO CLINIC AND THE MAYO FOUNDATION, ROCHESTER, MINNESOTA

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THE EFFECT OF BORIC ACID ON THE GROWTH OF TOBACCO PLANTS IN NUTRIENT SOLUTIONS

T. ROBERT SWANBACK¹

(WITH SIX FIGURES)

Although it is quite possible to grow tobacco in all parts of America and in most of the countries of the world, it is well known that only a few restricted regions produce the world's supply, and attempts to establish the industry in new sections with few exceptions have ended in failure. Apparently there is something in the soil or possibly in the climate of these tobacco growing sections, which is favorable for the development of tobacco of a quality which the trade desires. Fertilizers are known to have a very marked effect on the quality of tobacco which is produced; for instance, chlorine in the fertilizer is not favorable to quality, while potassium is decidedly so. There is considerable knowledge concerning the effect of some of the major elements in the nutrition, but it is not sufficient to explain altogether the peculiar behavior of tobacco on different soils and under different fertilizer treatments. Although it has long been suspected that some of the elements may have a decided influence in this respect, practically no experimental work with them has been recorded.

The present investigation was undertaken with the object of determining the influence of one of these minor elements, *viz.*, boron, in the nutrition of tobacco. Similar studies of boron on other plants have indicated that it is of considerable importance; but, with one exception, tobacco has not been included among the plants used in such investigations.

In this paper the effects of boron on plants when grown in chemically pure water culture solutions are under consideration.

Review of literature

COLLINGS (11) in a review of literature mentions that as early as 1857 German workers reported the presence of some boron in seed of *Maessa picta* and furthermore that the element later on was found to be present in more than fifty miscellaneous plants. AGULHON (1) found 0.2 per cent. of boron in the ashes of birch. In experiments on growth of different plants he found that the effect of boron in increasing concentrations could be ex-

¹ The writer wishes to express his appreciation for the kindly help and suggestions received from Messrs. O. L. CLARK, L. H. JONES and A. B. BEAUMONT, of the Massachusetts Agricultural College, and Dr. P. J. ANDERSON, of the Tobacco Experiment Station, Windsor.

pressed by a curve with a very characteristic optimum. He asks: "Is higher plant life possible in the absence of boron?" On the other hand, he found that 200 gm. of boric acid per liter rendered growth impossible.

Rothamsted experiments (9) show that boric acid is definitely poisonous to barley at a concentration of 4 parts per million. Frequently a depressing effect was evident at much smaller concentrations. Peas could withstand 20-40 parts per million. In concentrations as low as 0.4 parts per million the effect of boron on barley was made evident by the death of leaves, although in many cases with this plant this may be about the critical minimum concentration where detrimental effects cease.

In the presence of boric acid the roots of plants remained in much healthier condition, which suggests that the acid has an antiseptic action and thus protects the roots. This is in agreement with AGULHON (1) who found that even in the case of small doses of boric acid there was apparently an antiseptic effect. BRENCHLEY (9) reports that in general boric acid is toxic down to 20 parts per million.

AGULHON (1) in sand cultures tried different concentrations of 0, 0.1, 1.0 and 50 parts of boric acid per million of nutrient solution. Using wheat as an indicator plant he found that the maximum concentration caused the leaves to turn yellow. In soil cultures the same investigator found that the toxic doses of boric acid approached in concentration those in nutrient solutions rather than those of sand cultures, and he assumed that the boric acid was fixed by the soil, probably as insoluble borate of calcium. He found that an excess of boron in the nutrient medium resulted in an excess of boron in the ash, and he concluded from this that the plants react to an excess of a useful element. In water cultures at concentration of 5-10 parts per million he obtained an increase of up to 30 per cent. in dry weight due to boric acid, and 0.1 parts per million of boric acid per liter of nutrient solution applied to soil gave an increase of 7.5 per cent. of dry weight.

ALBANO (2) in a study of various growth stimulants such as borax, manganese, and zinc concludes that application of these elements in dilute form accelerates growth sufficiently to make their use financially profitable.

ANDOUARD (3) reports that an application of 30 kilograms of boric acid per hectare had no effect upon oats, wheat, kidney beans, potatoes, clover and turnips. BLAIR (6) comes to a somewhat similar conclusion when he reports that 30 pounds of borax per acre had only a slightly depressing effect on potatoes. Fifty pounds of borax per acre had only a slightly depressing effect when applied in drill *three weeks* before planting. This seems to be in agreement with AGULHON's conclusion, that borax probably changes in the soil to an insoluble calcium borate, thus preventing the detrimental effect on plant growth.

MORSE (15) found that an application in drill of 4.4 pounds per acre caused severe injury to beans, while the double amount (8.8 pounds) broadcast caused no apparent injury to oats, wheat, and buckwheat. BRECKENRIDGE (8) in experiments with corn and beans observed a toxic effect from borax applied at a rate of 6 pounds per acre, and 10 pounds shows decidedly harmful effects. NELLER and MORSE (17) found that 3 pounds of borax to the acre was the largest amount that could be applied in drills with safety to beans, and the limit for corn seemed to be about 5 pounds and for potatoes somewhat higher. It is apparent that these last mentioned investigators have been dealing with maximum applications of borax, where this salt was neither injurious nor beneficial for the crops. Except for the South Carolina report (21), that lime did not prevent injury from borax, one might assume that AGULHON's theory on formation of insoluble calcium borates in the soil still holds: *viz.*, that the soils might not have contained sufficient amounts of CaO to fix the surplus borax. Other investigators such as CONNER (12), SCHREINER (19) and co-workers, PLUMMER and WOLF (18), have all found injurious effects on crops even with small applications of borax. PLUMMER and WOLF (18) are among the few investigators who include the tobacco plant in the study of the effects of borax upon growth. They report that in sandy soils as little as 1 pound anhydrous borax per acre injured tobacco.

AGULHON (1) again, speaking of the difficulty of studying boron effects in culture media entirely free from that element, because of traces of boron in the seeds, reports that addition of boron to nutrient solutions gives increases sometimes reaching about 60 per cent.

MAZÉ (14), experimenting with various elements in respect to effect on plant growth, states that results apparently show that the elements used (aluminum, boron, fluorin, iodine and arsenic) are all necessary for the best growth of maize, with the exception of arsenic. BERTRAND (5), on the other hand, adding manganese to the group of favorable plant stimulants, could not obtain any beneficial effect of boric acid.

NAKAMURA (16) found that one milligram of borax per kilogram of soil had evident stimulating effect on the growth of peas as observed by increase in height of shoot over the control plants. In water culture the increase amounted to as much as 30 per cent.

WARINGTON (22) has given experimental evidence to prove that Windsor beans require small amounts of boron for normal development. BRENCHLEY (10), in later work with boron as affecting the nodule formation on the same kinds of beans, found that in the absence of boron the vascular supply of the nodule is defective and in plants receiving no boron the number of nodules attaining microscopic size is much reduced as compared with normal plants.

BÖSEKEN (7) *et al.* found that certain fungi were depressed by 0.06 per cent. boric acid, while for *Aspergillus niger*, for example, it required 0.5 to 1.0 per cent. of this acid for a similar effect. The authors conclude that the deleterious effect of boric acid and other compounds in the nutrient solutions is perhaps attributable to selective combinations.

SOMMER and LIPMAN (20) finally obtained evidence to show that boron is indispensable to the normal growth of green plants. From their thorough study of the effect of boron on various plants they conclude that as little as *one half part per million* of boron is sufficient in most cases to provide normal growth.

Experimental

The experimental work as reported here was undertaken with the object of answering the following questions: (1) If boron is necessary for normal plant growth, how does the tobacco plant react to boron treatment? (2) If boron has a favorable effect upon the growth of the tobacco plant, what concentration gives the optimum growth?

METHODS

The first part of this experiment was carried out with nutrient solutions as culture media, using SHIVE'S (13) three-salt solution (R_5C_2) having the following partial-volume molecular concentration:

KH_2PO_4	0.0180 or 2.450 gm.
$Ca(NO_3)_2 \cdot 4H_2O$	0.0052 or 1.228 gm.
$MgSO_4 \cdot 7H_2O$	0.0150 or 3.698 gm.
H_2O	1,000 gm.

Iron, added in the form of $FeSO_4$, equals 0.0008 gm.

Boric acid in solution was added to obtain the following concentrations: 0.0, 0.5, 1, 2, 4, and 50 parts per million.

Two jars were used for each concentration, and three tobacco plants were planted in each jar. After two weeks they were thinned out to one in each.

RESULTS

If boron in the form of boric acid does affect the plant growth, the plants in the earlier stages of development should very likely give noticeable response to different concentrations of the element. The dry weights of plants removed in thinning is shown in table I.

From these preliminary yield figures it is evident that boron had a different effect at the individual concentrations of the element. The difference between the check, however, and the 0.5 ppm. is not significant. Of the different concentrations 1 ppm. seemed to give the best results in earlier

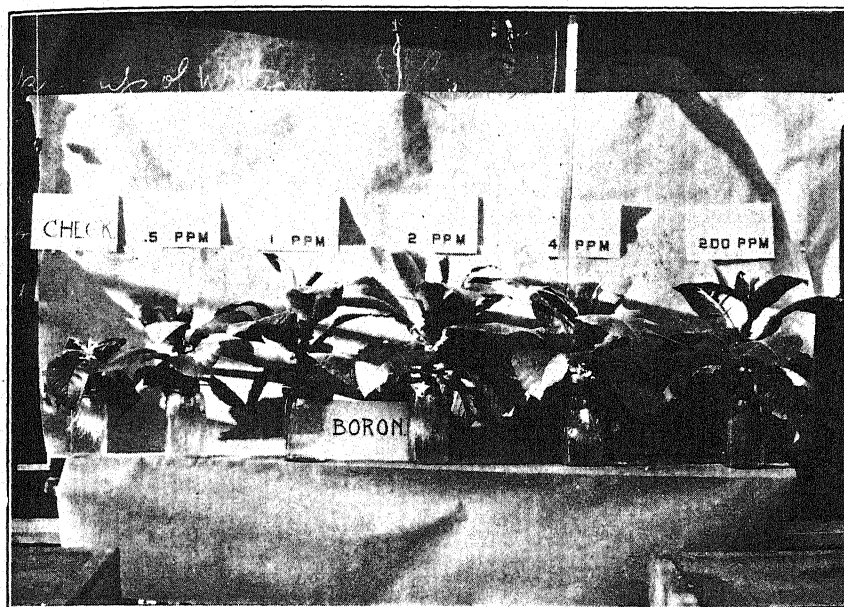


FIG. 1. Optimum concentration of boric acid for normal development of tobacco plants. Sixty days growth. Concentrations indicated in the photograph.

TABLE I

WEIGHT OF PLANTS REMOVED IN THINNING AFTER 14 DAYS

JAR No. OF	CONCENTRATION OF H_2BO_3	DRY WEIGHTS			RELATIVE WEIGHT OF AVERAGE PLANT
		2 PLANTS	4 PLANTS	AVERAGE PER PLANT	
	ppm.	gm.	gm.	gm.	
Check A	0.0	0.892			
Check B	0.0	0.887	1.78	0.445	1.00
1	0.5	0.882			
2	0.5	0.727	1.61	0.403	0.90
3	1.0	0.866			
4	1.0	0.827	1.70	0.425	0.95
5	2.0	0.810			
6	2.0	0.663	1.47	0.368	0.83
7	4.0	0.738			
8	4.0	0.917	1.66	0.414	0.93
9	50.0	0.750			
10	50.0	0.560	1.31	0.328	0.73

stages. Very noticeable is the sudden drop in yield when the concentration is doubled. This fact has been observed by other workers in this field.



FIG. 2. Optimum growth and development of leaves at a concentration of 2 ppm. of boric acid. Ninety days' growth. Concentrations indicated in the photograph.

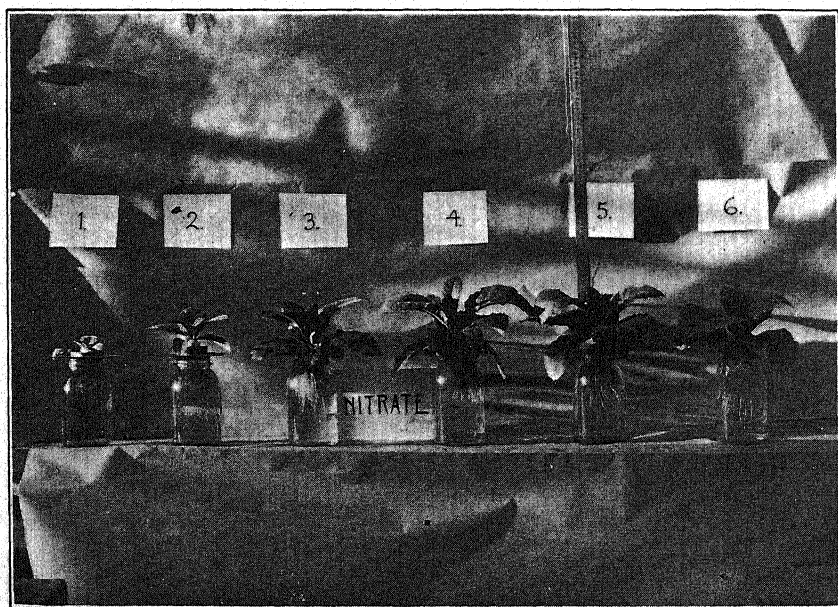


FIG. 3. Optimum growth at a concentration of 9 ppm. of nitrate nitrogen (no. 4). Plants about 40 days old.

No. 1, no nitrate; no. 2, one part per million of $\text{NO}_3\text{-N}$; no. 3, 3 parts per million; no. 4, 9.5 ppm.; no. 5, 31.5 ppm.; no. 6, 105 parts per million of $\text{NO}_3\text{-N}$.

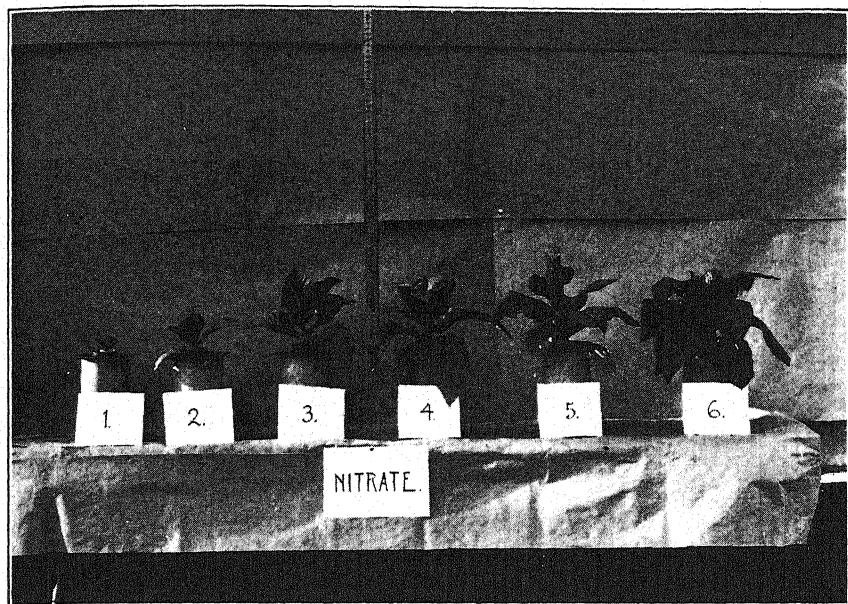


FIG. 4. Stunted growth of plants in nitrate series due to lack of boron. Plants 70 days old.

No. 1, no nitrate; no. 2, one part per million of NO_3 ; no. 3, 3 parts per million; no. 4, 9.5 ppm.; no. 5, 31.5 ppm.; no. 6, 105 parts per million of NO_3 .

The 50 ppm. concentration gave only slightly less yield than 2 ppm. and the plants did not show any injury either on the leaves or on the roots.

Figure 1 shows the plants after a growth period of 60 days. From this it is easily seen that the optimum growth is at the concentration of about

TABLE II
DRY WEIGHT OF PLANTS AFTER 60 DAYS OF GROWTH

NO. OF JAR	CONCENTRATION OF H_2BO_3	DRY WEIGHTS			RELATIVE WEIGHT OF PLANT
		TOPS	ROOTS	TOTAL WEIGHT	
	ppm.	gm.	gm.	gm.	
Check B	0.0	3.3	0.5	3.8	1.00
2	0.5	9.4	1.3	10.7	2.81
4	1.0	9.8	2.2	12.0	3.16
6	2.0	11.1	1.9	12.0	3.16
8	4.0	9.5	1.5	11.1	2.91
10	200.0	11.1	1.5	12.6	3.31



FIG. 5. Plants in nitrate series after application of boric acid, resulting in renewal of top growth.

No. 1, no nitrate; no. 2, one part per million of $\text{NO}_3\text{-N}$; no. 3, 3 ppm.; no. 4, 9.5 ppm.; no. 5, 31.5 ppm.; no. 6, 105 parts per million of $\text{NO}_3\text{-N}$; plants have in addition two parts per million of H_3BO_3 .

2 ppm. of H_3BO_3 . Half of the plants were harvested at this period of their development, while the rest were left to continue growth.

Table II shows the yield figures from this harvest.

From this table it is evident that the favorable concentration of H_3BO_3 lies around 2 parts per million. The check plant, as may be seen in figure 1, showed stunted growth, and the weight amounted to about one third of the lowest yield for boron-treated plants.

After 90 days growth the rest of the plants in the series were photographed and harvested (fig. 2). Here optimum concentration of 2 parts per million of H_3BO_3 is still more pronounced. All the plants had reached the budding or flowering stage. The largest leaf development, and somewhat delayed flowering, was shown by the 2 parts per million treatment. The yield figures in this case are shown in table III.

From this table it is found that 2 parts per million of H_3BO_3 is a decidedly favorable concentration for the normal growth and development of tobacco plants in water cultures.

In order to study the effect of higher concentrations of H_3BO_3 the highest concentration (50 ppm.) was doubled after the lapse of every two weeks

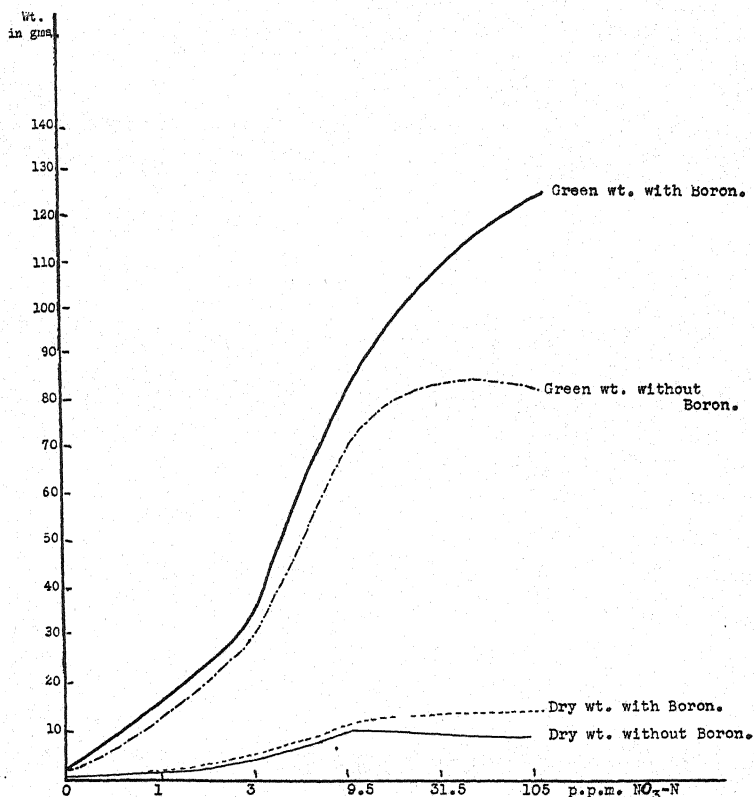


FIG. 6. Growth influenced by varying NO_3 concentrations and with boric acid added.

until it reached 400 parts per million. At this concentration the plants still grew normally and fruited, though the lower leaves dried off and the plant showed a yellowish green color.

TABLE III

DRY WEIGHTS OF TOBACCO PLANTS AFTER 90 DAYS

No. OF JAR	CONCENTRATION OF H_3BO_3	DRY WEIGHTS			RELATIVE WEIGHT OF PLANT
		TOPS	ROOTS	TOTAL WEIGHT	
	ppm.	gm.	gm.	gm.	
Check A	0.0	10.5	1.5	12.0	1.00
1	0.5	22.5	5.2	27.7	2.30
3	1.0	30.0	6.0	36.0	3.00
5	2.0	36.2	7.5	43.7	3.64
7	4.0	33.0	4.7	37.7	3.14
9	400.0*				

* This plant saved for maturity of seeds.

TABLE IV
WEIGHT OF PLANTS WITH 2 PARTS PER MILLION OF H_3BO_3 AND WITHOUT BORON
TREATMENT

TREATMENT AND NUMBER	CONCENTRATION OF NO_3	DRY WEIGHT			RELATIVE WEIGHT OF PLANTS
		TOPS	ROOTS	TOTAL WEIGHT	
Check, average of two plants	ppm. 0.0	gm.	gm.	gm.	
Boron A 1	1.0	1.0	0.4	1.4	1.0
" A 2	1.0	1.6	0.4	2.0	7.0
" B 1	3.0	4.0	1.4	5.4	10.0
" B 2	3.0	4.0	1.2	5.2	27.0
" C 1	9.5	9.3	2.4	11.7	26.0
" C 2	9.5	9.3	1.9	12.2	58.5
" D 1	31.5	10.6	3.0	13.6	61.0
" D 2	31.5	10.8	2.8	13.6	68.0
" E 1	105.0	12.1	2.7	14.8	68.0
" E 2	105.0	11.5	2.7	14.2	74.0
					71.0
No boron A 3	1.0	0.6	0.15	0.75	3.8
" " A 4	1.0	1.4	0.5	1.90	9.5
" " B 3	3.0	4.1	1.1	5.2	26.0
" " B 4	3.0	4.0	1.0	5.0	25.0
" " C 3	9.5	8.3	2.0	10.3	51.5
" " C 4	9.5	8.4	2.0	10.4	52.0
" " D 3	31.5	9.1	1.8	10.9	54.5
" " D 4	31.5	7.4	2.0	9.4	47.0
" " E 3	105.0	10.0	2.2	12.2	61.0
" " E 4	105.0	7.0	1.5	8.5	42.5

In a separate experiment [on nitrate concentration, following the method of ARRHENIUS (4)], the effect of boric acid in nutrient solutions was studied. The optimum concentration of nitrate nitrogen was determined (as seen in figure 3, jar no. 4) but later the plants seemed unable to develop any further. The top leaves curled and turned slightly brownish. From the observations on the boron experiment it was thought well to try the 2 parts per million concentration of H_3BO_3 as a means of promoting further growth. The treatment was applied to half the plants and the whole series was continued for a month. After the first week it was noticed that those plants treated with boron renewed the top growth and the results after a month may be seen in figure 5. Figure 4 shows plants of the same age without boric acid. In this case they have not increased much in size, while the former shows a very different curve of growth, as may be seen in fig. 6.

Comparative yields of the plants with and without boron treatment are presented in table IV.

Summary

The effect of boric acid has been studied with respect to tobacco and as applied to nutrient solutions. The behavior of the plants indicated that boron is an essential element in plant nutrition and causes slight injury to plants at a gradually increased concentration up to 400 parts per million. Furthermore, addition of boric acid to nutrient solutions, in which plants were unable to continue top growth because of the lack of boron enabled the plant to re-establish the growing point.

Two parts per million of boric acid in the nutrient solution, corresponding to about four tenths parts per million of boron, gave optimum growth.

MASSACHUSETTS AGRICULTURAL COLLEGE,
AMHERST, MASSACHUSETTS.

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NOTES ON APPARATUS FOR LOW TEMPERATURE RESPIRATION STUDIES*

J. H. BEAUMONT, J. J. WILLAMAN AND W. A. DELONG

(WITH SIX FIGURES)

In the series of studies on respiration of apple twigs in relation to winter hardiness which is being conducted at this institution, apparatus which involves certain novel features and combinations of old features, has been used with such success as to induce the writers to publish a brief description of the various set-ups.

The apparatus is most easily shown in the form of diagrams. Figures 1, 2 and 3 indicate in succession the air train through the various parts of the apparatus, and figure 4 is a wiring diagram. The legends, given in connection with each figure, describe the various parts.

Thermostat

During the first winter's work¹ a double-walled wooden box, about 12 x 12 x 45 inches was used as a thermostat, the outdoor temperature serving as a source of cold. One side of the box was a detachable door. One end was closed only by a piece of canvas tacked around the ends. Several branches of a tree could be inserted into the box through the open end and the canvas, together with some cotton wool, was tied around the branch for insulation. The branches were enclosed in tubes similar to 15, fig. 2, but of smaller diameter and sealed with a split rubber stopper and vaseline. The box was heated by electric globes, and the air stirred by a fan, the shaft of which extended through the end of the box opposite the canvas.

Because of the uncertainty of out-door temperatures it became necessary to employ artificial refrigeration. For this purpose a commercial ice-cream cabinet, such as is used behind the retail counter, proved to be very satisfactory. It contains six cells, each about 25 cm. in diameter and 50 cm. deep. The machine itself has a regulator which controls the temperature of the liquid in the tank (in this case alcohol), and which can be set at any temperature down to about -30° C. The temperature in each cell is kept constant by means of individual controls and by suitable asbestos insulation.

* Published with the approval of the Director as Paper no. 720, Journal Series, Minnesota Agricultural Experiment Station.

¹ BEAUMONT, J. H., and WILLAMAN, J. J. Preliminary report on the respiration of apple twigs during the winter. Proc. Soc. Hort. Sci. 1924: 99-104.

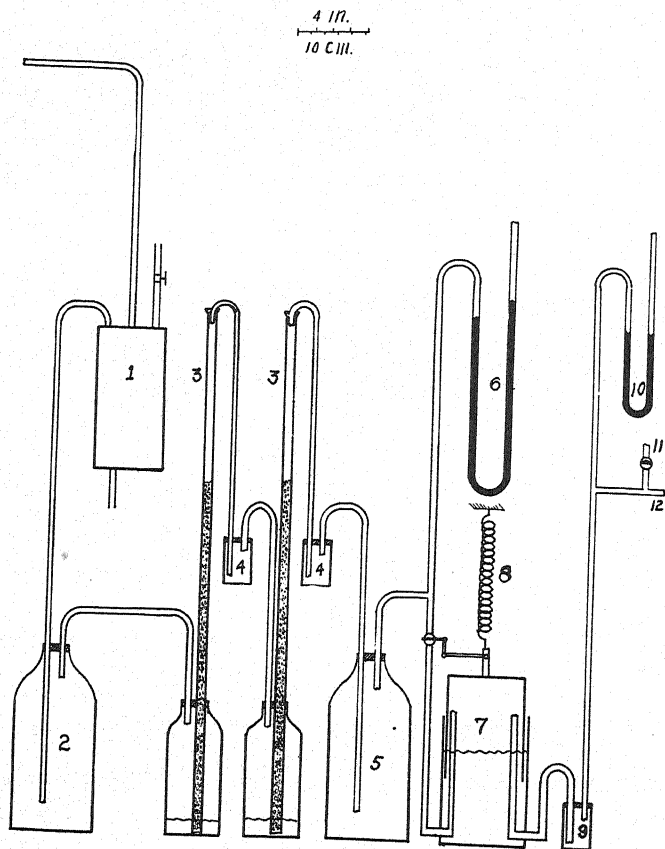


FIG. 1. Air washing section of respiration apparatus.

1. Water pressure pump. 2. Reservoir for air pressure. 3. Bead towers for washing CO_2 from air. 4. Catch-alls. 5. Reservoir for clean air. 6. Mercury manometer for indicating reserve air pressure. 7. Telescoping gas pressure regulator loaded with oil or water. 8. Adjustable spring supporting the cap of the regulator. 9. Oil trap. 10. Water manometer for indicating pressure in the respiration system. 11. Short circuit to 36, figure 3. 12. Connection to 13, figure 2.

A cross section of the cell, with all its equipment, is shown in figure 2. The drawing and the explanatory legend will make the set-up clear without further description.

With this cabinet it is possible to have each of the six cells at a different temperature. Their range in the writers' experiments has been from -10°C. to $+10^\circ\text{C.}$ By varying the amount of insulation in the cells undoubtedly a much greater range is possible.

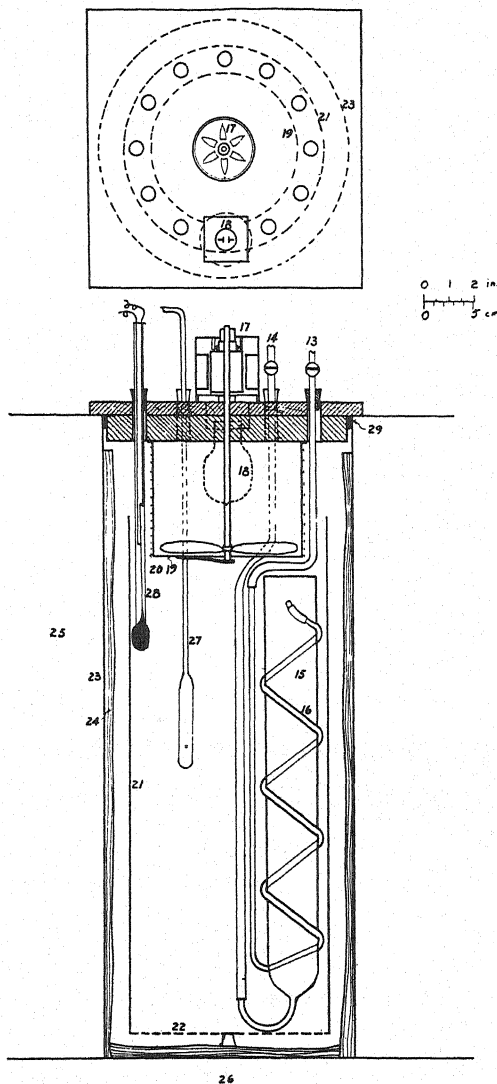


FIG. 2. Cell in ice-cream cabinet used as source of cold.

13. Tube for ingoing air. 14. Tube for outgoing air: connects with 30, figure 3. 15. Glass tube, one type of respiration chamber. 15a. (Figure 5.) Respiration chamber over plant pot, 15b. 16. Coil of copper tubing for cooling air before it enters respiration chamber. 17. Fan motor. 18. Socket and light, for source of heat. See figure 4 for wiring. 19. U-shaped iron strap for holding fan guard and carrying the bearing for the fan shaft. 20. Cylindrical wire screen for fan guard. 21. Cylinder of sheet metal. 22. Screen bottom of 21, kept about 3 cm. above bottom of cell. 23. Wall of the cell of the freezing cabinet. 24. Asbestos insulation of cell. 25. Fluid of the freezing cabinet. 26. Cork insulation of the freezing cabinet. 27. Recording thermometer bulb. 28. Thermoregulator. See figure 4 for wiring. 29. Ring of felt.

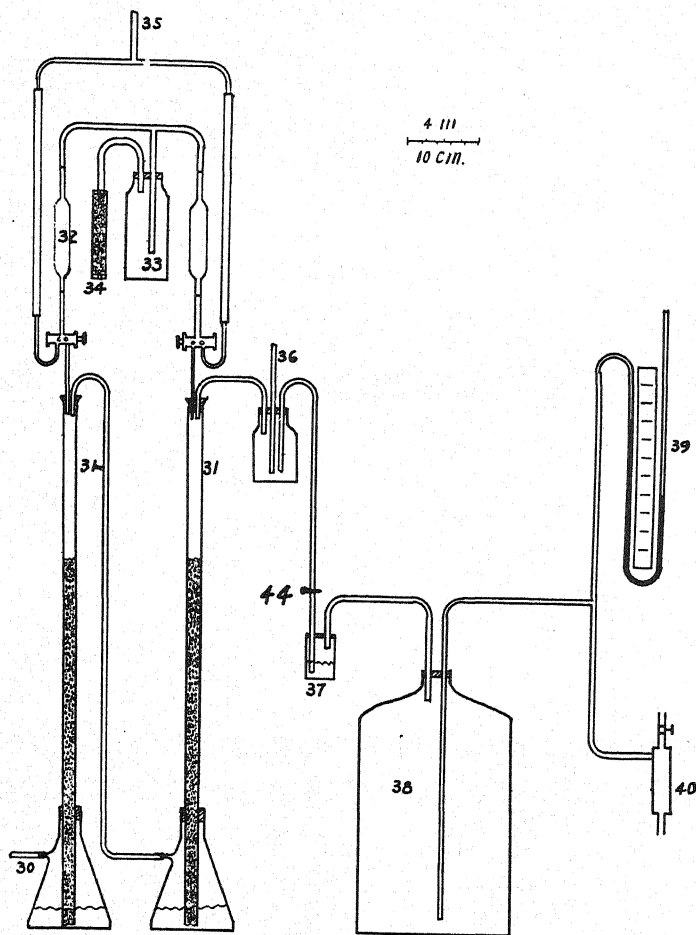


FIG. 3. CO_2 measuring section of respiration apparatus.

30. Connection to 14, figure 2. 31. Bead towers containing standard $\text{Ba}(\text{OH})_2$. 32. Pipettes for measuring $\text{Ba}(\text{OH})_2$. 33. Reservoir of CO_2 -free air. 34. Soda-lime tube. 35. Connection to reservoir of standard alkali. 36. Short circuit to 11, figure 1, for diverting air away from respiration system. 37. Bubbling indicator of speed of air. 38. Reservoir of vacuum. 39. Mercury manometer for indicating amount of suction. This was kept at 4 inches. 40. Water suction pump.

Respiration chamber

The most efficient shape of respiration chamber is one built according to the "stream line" principle, so that the shortest possible time will be required for sweeping out the contained CO_2 . The chamber shown at 15 in figure 2 has been found by the writers to be admirable for twigs, seeds, and

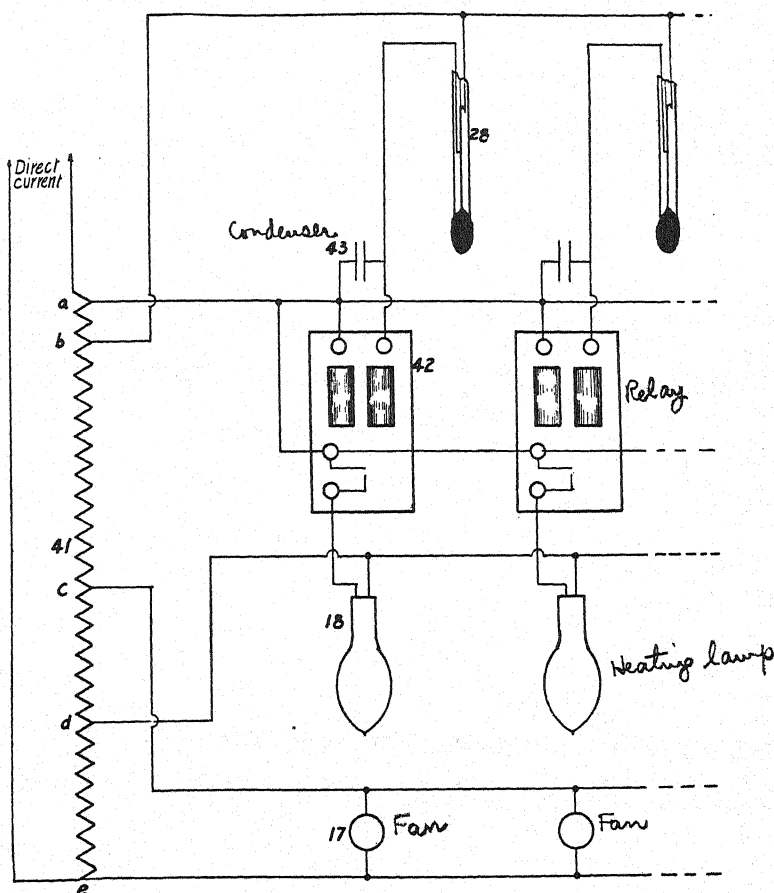


FIG. 4. Wiring diagram of controls for apparatus in fig. 2.

41. Coil of resistance wire. Shunt a-b supplies the thermoregulators; a-d supplies the lamps; c-e supplies the fans. Each of these sets of equipment is wired in parallel. The shunt ratios shown are not intended to be exact. 42. Relays. 43. Condensers.

tubers.² Aspiration at the rate of 18 liters of air per hour will sweep this chamber in less than 10 minutes. Another type of chamber that will fit into the same cell is the one illustrated in figure 5, which was used by MARTIN³ with wheat plants. It is made of sheet metal, and sets on the soil in an earthenware jar containing the plants. The soil is covered with melted vaseline.

² WILLAMAN, J. J., and BEAUMONT, J. H. Effect of accumulated carbon dioxide on plant respiration. *Plant Physiol.* (In the Jan. 1928 number—Ed.) (In press.)

³ MARTIN, J. H. Comparative studies of hardiness in wheat. *Jour. Agr. Res.* (In press.)

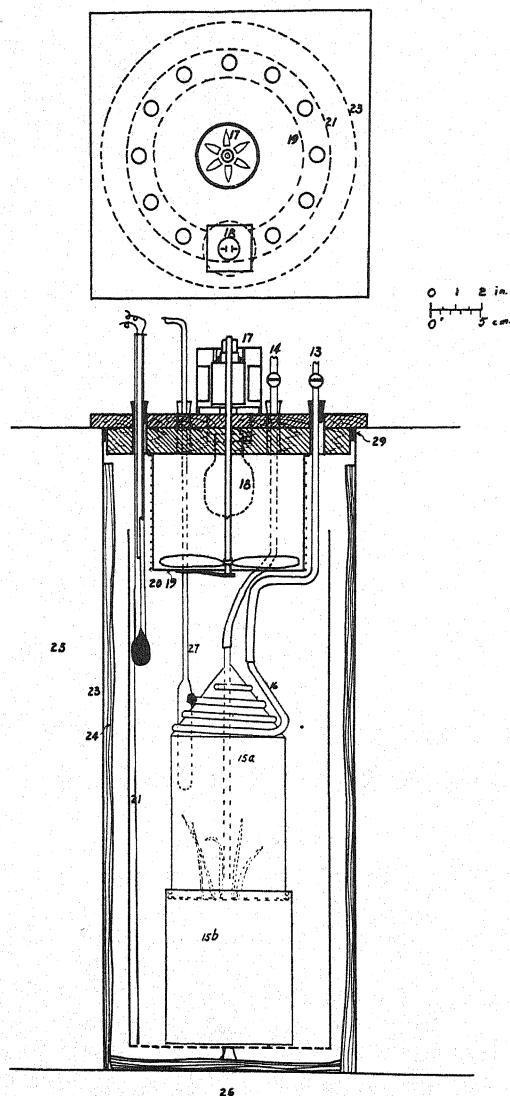


FIG. 5. Same as fig. 2, except for the different type of respiration chamber.

Absorption of CO_2

The writers are of the opinion that the simplest and most effective type of absorption apparatus is the bead tower, arranged more or less like

GURJAR'S.⁴ It can be used with 50 per cent. KOH for washing the air entering the respiration chamber, and with more dilute alkali for the CO_2 to be measured. In figure 1 two towers for washing the air are shown although in some cases the writers used concentrated H_2SO_4 in the second one to dry the air to avoid formation of ice in the tubes at the lower temperatures.

The strength of alkali to be used in the measuring towers is governed by the amount of CO_2 expected. A convenient strength is one that is roughly equivalent to a 0.04545 N. acid, since one cc. of the latter is equivalent to one mg. of CO_2 . A tower 2 cm. in diameter and one meter long will conveniently receive a charge of 50 cc. Not more than two-thirds of this may, with safety, be neutralized by the CO_2 . Flexibility is secured by using two towers in series according to the amount of CO_2 expected, in which case the contents of the two flasks were mixed and titrated.

NaOH is more satisfactory than $\text{Ba}(\text{OH})_2$, because of the precipitate of BaCO_3 . When the former is used, neutral BaCl_2 solution should be added just before titration, to precipitate the carbonate. Then a single titration with phenolphthalein will suffice.

It has been found that, unless the laboratory air be heavily charged with CO_2 , the beads can be dropped into the flask, the tower rinsed with CO_2 -free water, and the titration conducted in the flask in the presence of the beads, without appreciable contamination from the air.

Another type of absorption vessel that has been used with success is the one shown in figure 6. The tube through which the air enters is open at

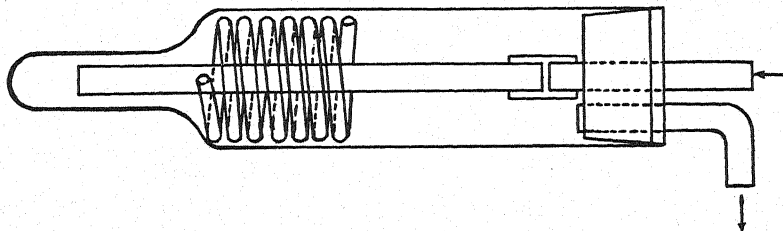


FIG. 6. Coil type of CO_2 absorption vessel.

the bottom. About 5 cm. above the bottom the coiled tube is attached. The vessel is filled with a measured quantity of alkali to about the top of the coil. As the air passes down the tube it enters the coil as a series of bubbles and pushes some of the liquid ahead of it. By adjusting the rate of flow of the air, a succession of gas bubbles ascending the coil is easily obtained. Very thorough washing of the air as well as circulation of the liquid is thus

⁴ GURJAR, A. M. The adaptation of TRUOG'S method for the determination of carbon dioxide to plant respiration studies. *Plant World* 20: 288-293. 1917.

attained. The advantage of this type of vessel is that it operates under a hydrostatic head of only about 4 cm. Its disadvantage is that there is a definite speed limit of aspiration of about 6 liters per hour, which, however, is sufficient for the ordinary small respiration chamber and for continuous aspiration. If this is exceeded, the air enters the coil too rapidly to allow the entrapping of liquid. This cell was found particularly useful in measuring the CO_2 produced by growing fungi.⁵ The cell is 5 cm. in largest diameter and 15 cm. in length. A somewhat similar type of cell, arranged for the electrical measurement of CO_2 , was used by HARVEY and REGEIMBAL.⁶

Circulation mechanism

It is generally considered desirable not to subject plant tissue to a pressure appreciably above or below atmospheric. Since any form of liquid absorption medium entails a certain hydrostatic head, it becomes necessary in such a case to employ pressure regulating devices. The system illustrated here consists of a water-operated blow pump, 1, figure 1, which pushes the circulating air through the washing towers, and a suction pump, 40, figure 3, which pulls the air through the measuring towers. By means of the regulator, 7, figure 1, and the pinch cock, 44, figure 3, the air through the respiration chamber flows at atmospheric pressure ± 2 cm. of water. The regulator was originally designed to control the flow of gas to a burner under an incubator. It is filled with oil or water. As the pressure inside increases, the telescoping cover rises and finally shuts off the entering air by means of the air connected to the valve in the tube carrying the entering air. By adjusting the spring holding the lid, the pressure required to close the valve can be regulated closely. The rate of aspiration can be kept very constant by maintaining the manometer, 39, on the suction line at a certain level.

The same combination of controls is successful also when an electrically driven blower is used for circulation of air. The type used by the writers was a rotary pump, with a connection in one side for suction and on the other for pressure. The latter was connected with 3, figure 1, and the suction with 37 or 31, figure 3. Thus the same air circulated continuously through the whole system except for harmless leaks in the pump. A rheostat was used to throttle the blower much below its usual speed. The speed of this machine is so regular that reservoirs 2, 5, and 38 can be dispensed with. By means of the above set-up as many as three respiration chambers have been operated from one pump and one pair of washing towers, by sim-

⁵ WHITE, MOLLIE G. The pentose metabolism of *Fusarium lini*. (In press, Biochem. Journ.)

⁶ HARVEY, R. B., and REGEIMBAL, L. O. A conductivity cell for continuous measurement of respiratory rate. Plant Physiol. 1: 205-206. 1926.

ply dividing the stream of clean air before it enters the chambers, and combining the streams which leave the measuring towers.

Heat control mechanism

An electric globe furnished the heat in the cell surrounding the respiration chamber. This was connected to a relay and to a HARVEY thermoregulator⁷ in the usual way. Since there were so many cells within a small space, a board containing the relays and condensers, and a long coil of resistance wire to furnish the various currents were found very convenient. The wiring diagram in figure 4 indicates how a number of complete units may be connected in parallel from this resistance coil, thus saving wiring and space.

UNIVERSITY OF MINNESOTA,
ST. PAUL, MINNESOTA.

⁷ HARVEY, R. B. A thermoregulator with the characteristics of a Beckmann thermometer. Jour. Biol. Chem. 41: 9-10. 1920.

THE DETERMINATION OF PEPTIDE AND BASIC FORMS OF NITROGEN*

Inasmuch as a previous section of these recommendations¹ has dealt with the more significant non-protein forms of nitrogen other than basic compounds the present treatment is limited to peptides and basic forms of nitrogen. The former include all stages of conjugation of amino-acids and the latter embrace all soluble forms which, by virtue of their precipitability, merit the designation "basic."

Proteins

So far as present information extends, this is the only group of nitrogenous compounds in plants which involves a serious issue relative to interpretation of solubility. It is quite to be expected that the degree of apparent solubility of such a colloid should be affected by the dispersive action of grinding in water for extraction. CHIBNALL (3) proposes a method of expressing the undiluted sap from cytolyzed tissue, which he believes isolates from the cytoplasmic proteins the naturally occurring proteins of the vacuolar fluid. The treatment offers a means of separating to a large degree the simpler nitrogenous constituents from the proteins. The degree of solubility, or more properly of colloidal suspension, of proteins, depends upon the character of the proteins, upon whether they occur free or combined, and upon mechanical interference of such other cell constituents as the celluloses of the wall. The globulins, prolamins and glutelins of seeds present special conditions of solubility which have not yet been found prominent in the proteins of vegetative organs. Their presence remains a possibility, in the event of which watery extraction may render globulins insoluble and alcoholic extractions may include prolamins. On the other hand, the presence of inorganic salts in plant sap may greatly affect the extraction of proteins. While the limited data extant indicate a high degree of water solubility of leaf proteins, the presence of glutelins in such proteins is by no means excluded. OSBORNE and his coworkers (12, 13) found that strong alcohol and dilute sodium hydroxide following successively a watery extraction of fresh alfalfa removed little nitrogen, while hot alcoholic alkali was very effective. The last-named treatment seemed to decompose complexes of

* Section V of the report of the Committee on Methods of Chemical Analysis of the American Society of Plant Physiologists: The complete set of five separates of this series of recommendations of the Committee may be obtained at 25 cents (cash preferred), post-paid in the United States. Address Dr. W. E. Tottingham, Agricultural Chemistry Building, Univ. of Wisconsin, Madison, Wisconsin, U. S. A.

¹ PLANT PHYSIOL. 2: 205-211. 1927.

protein with pigments. The writer and his associates (17) recovered little protein by alcoholic alkali and believed the large residue of insoluble nitrogen could be accounted for by occlusion of protein within the structural carbohydrates of the cell wall. On the other hand, JONES² is led by nutritional studies with wheat bran to the conclusion that the rôle of wall materials in reducing solubility has been exaggerated and that indigestibility is more often due to characters of the protein itself.

Wide latitude must be allowed for variability of status of proteins in different species. Furthermore, too much emphasis hardly can be placed upon the possible denaturing effects involved in extraction and recovery of proteins from the living cell. GORTNER³ stresses the possibility of irreversible coagulation of soluble proteins due to supersaturation in interfacial films, enhanced by access of air during extraction.

In view of the lengthy process involved in the extraction of cell proteins with water, an index of practical termination of the process must be rather arbitrarily selected. Perhaps the decline of coagulable matter in the filtrate to a low, constant level is the most feasible index. As mentioned in an earlier section of these recommendations, colloidal protein sols require modification of the usual practice in filtration. The significance of quantitative distinctions between soluble and insoluble protein is rendered decidedly problematical, at least with vegetative tissues, by lack of information regarding variability of both character and function of the compounds involved. If the method of watery extraction or the use of any other solvent is adopted, the limitations imposed should be recognized. CHIBNALL⁴ believes that such modifying treatments as saponification by alcoholic alkali will have limited significance in general usage for protein extraction unless one can limit the forms concerned.

The control over enzymatic hydrolysis which is attainable by extraction with alcohol has led to common use of this reagent for separating protein nitrogen from non-protein forms. OSBORNE and his associates (12, 13) found that about 50 per cent. alcohol was sufficiently concentrated for this purpose with green alfalfa. It is preferable to make a rapid watery extraction before precipitating the proteins, thus avoiding occlusion of solutes by coagulation in the tissue. When the concentration of alcohol is raised to 80 per cent. as advised earlier in these recommendations,⁵ the efficiency of the solvent for non-protein nitrogen may be reduced, necessitating increased washing. If alcohol is applied directly to the cut tissue, grinding treatment should be eventually applied. BURRELL⁶ advises drying

² JONES, D. BREESE. Bur. Chem., U. S. Dept. Agr. Private communication.

³ GORTNER, R. A. Dept. Agr. Biochem., Univ. of Minnesota. Private communication.

⁴ Private correspondence.

⁵ PLANT PHYSIOL. 1: 399-400. 1926.

⁶ BURRELL, R. C. Dept. Agr. Chem., Ohio State University. Private communication.

the residue from extracted fresh tissue at 105° C., then pulverizing and reextracting with alcohol by percolation to a colorless extract, as practiced by THOMAS and DUTCHER⁷ with sugar extraction. Only in rare cases will the use of a special factor, in preference to the conventional 6.25, for converting nitrogen as determined by the KJELDAHL method to protein be justified. In any case, the use of this factor involves the assumption that all of the nitrogen so determined belongs to protein.

If watery extraction is practical the protein may be either precipitated with the insoluble matter of the tissue for determination as total protein or it may be separated therefrom. Cupric hydroxide in the form of STUTZER's reagent, which was formerly used almost exclusively for this separation, does not eliminate lower forms of nitrogen, as shown by HART and BENTLEY (6), Miss O'DWYER (10) and others. CHIBNALL (2) coagulated watery extracts of bean leaves merely by heating to 60° C., but some types of tissues require acidulation at the boiling-point for complete flocculation of the soluble protein. Also, such added materials as calcium carbonate may upset the normal conditions of solubility and precipitation.

Acetic acid has been most commonly employed for coagulation, as an excess is less likely to hold protein in solution than with the stronger mineral acids. A few drops of 50 per cent. acetic acid usually suffice to confer distinct acidity on 500 cc. of extract, equivalent to 25 gm. of fresh tissue, and to accomplish sharp coagulation. CHIBNALL⁸ adjusts the pH of the extract to 4 or 5, thus favoring the insolubility at the isoelectric range as determined for some plant proteins. Filtration is generally rapid on fluted funnels and the coagulum may be well washed with boiling water. A correction should be made, of course, for nitrogen in the filter paper.

In recent years other precipitating agents are displacing acetic acid. HILLER and VAN SLYKE (7) found that tri-chloroacetic acid precipitated blood proteins uncontaminated by proteoses. GREENWALD (5) used 0.25 per cent. of this reagent and MERRILL (9) found this concentration as effective as more. Due to its greater strength less is required than of acetic acid, while its decomposition during boiling renders an excess unobjectionable. APPLEMAN⁹ reports satisfactory results from its use. THOMAS (16) recommends colloidal iron as a protein precipitant.

Proteoses and simpler peptides

Excepting special cases where their occurrence may bear unusual significance it is not desirable to distinguish proteoses or peptones from soluble proteins. Thus, the generally small amounts of these nitrogenous fractions may be precipitated in common with protein by means of tungstic

⁷ Jour. Amer. Chem. Soc. 46: 1666. 1924.

⁸ Private communication.

⁹ APPLEMAN, C. O. Dept. Plant Physiol., Univ. of Maryland. Private communication.

acid. FOLIN and WU (4) prefer this reagent to tri-chloroacetic acid in blood analysis and RUMSEY (15) used it effectively in flour extracts when the pH was adjusted to 2.0 or less. MERRILL (9) found a somewhat higher optimal pH in work on bacteriological sera. Successful use of it on plant sap and pollen extracts is reported by GORTNER.¹⁰ When proteoses are to be determined it does not seem advisable to distinguish primary from secondary forms as in past biochemical practice. CHIBNALL (2) precipitates this fraction by saturating with zinc sulphate in acid solution, washing with this reagent, dissolving in water and reprecipitating. For the precipitation of proteoses and peptones in flour digests OLSEN and BAILEY (11) used stannous chloride in faintly acid solution followed by cupric hydroxide formed *in situ*. According to BLISH (1) the latter is effective down to simple peptides. The latter can be determined in the filtrate by the increase of α -amino nitrogen resulting from hydrolysis. The common practice in this respect is exemplified by the work of JODIDI (8), save that buffer constituents necessitate determining amino-nitrogen by VAN SLYKE's method as given in a previous section of these recommendations.

Basic forms

As stressed by CHIBNALL,¹⁰ meagerness of knowledge renders unwise the drawing of empirical distinctions between analytical groups, and this applies with particular force to "basic nitrogen." It has been common practice to isolate this group by precipitation with phosphotungstic acid in weakly acid solution (5.0 per cent. H_2SO_4 or 2.5 per cent. HCl). This results in throwing a mixture of forms of nitrogen compounds together, more particularly a variety of cyclic bases and the diamino-acids. VAN SLYKE (18) gives a procedure for decomposing this precipitate in alkaline solution with barium hydroxide and fractionating the diamino-acids, but this should hardly be attempted by inexperienced analysts. Moreover, the method applies only to the diamino-acids obtained by the hydrolysis of proteins. It is more feasible to determine the total nitrogen and amino nitrogen contents in different aliquots of the solution containing the basic nitrogen. PLIMMER and ROSEDALE (14) give directions for overcoming difficulties here in the estimation of arginine. CHIBNALL uses phosphotungstic acid under standard conditions, without partitioning the precipitated nitrogen. As emphasized by both VAN SLYKE¹¹ and VICKERY,¹¹ one must consider the possible variability of makeup in this portion of the nitrogenous constituents, although its amount is often too small to permit fractionation.

In this connection the recent work of VICKERY (19, 20, 21, 22, 23, 24) is significant. He employs NEUBERG's alkaline mercurial reagent to precipitate amino nitrogen from protein-free extracts, from which precipitate a

¹⁰ Private communication.

¹¹ Private correspondence.

further fractionation of basic substances can be made by the use of phosphotungstic acid. This method seems to offer a sharper separation than was permitted by the use of phosphotungstic acid directly, when the fractions are subjected to KJELDAHL's method of determining nitrogen.

From the preceding brief survey it is apparent that while various separations can be accomplished the treatment of the complex mixture of basic forms of nitrogen in plant extracts, even by proximate methods of analysis, is hardly feasible for general practice. When experienced investigators in this field freely admit lack of regular procedure the novice analyst must be content to await their further progress. Meanwhile, he can only apply with caution proximate separations of the sort here mentioned, interpreting his results in a corresponding conservative spirit.

This report was organized by W. E. TOTTINGHAM for the Committee.

C. O. APPLEMAN

W. E. LOOMIS

T. G. PHILLIPS

W. E. TOTTINGHAM (chairman)

J. J. WILLAMAN

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BRIEF PAPERS

THE APPLICATION OF PHYSIOLOGICAL METHODS TO WEED CONTROL

Convolvulus arvensis, commonly known as wild morning-glory or bind-weed, is the most noxious weed pest in orchards and fields of California and other western states. No satisfactory method has been devised for its control or eradication. It occupies the most fertile soils and the area of infestation increases every year.

We have been unsuccessful in our efforts to control it by the methods usually recommended for the control of perennial weeds, *viz.*: keeping the stems cut below the surface of the ground. This failure is due to the large storage capacity of the root and its ability to produce new shoots. Experiments in which the stems have been kept from appearing above the ground for three years have not given satisfactory results. GRAY¹ states that roots taken from a depth of 14 feet are capable of producing new plants. Roots have been found at even greater depths.

The plants normally possess a tap root with many lateral branches. These roots are capable of producing shoot buds which develop into a complex network of rhizomes permeating the soil from a considerable depth. These rhizomes appear at the surface at irregular periods as leafy shoots. The cutting of the roots stimulates the production of many buds which in turn increase the number of rhizomes and leafy shoots. Cutting with a weed-cutter, when the soil moisture is abundant, tends to spread the severed parts over the field where they may grow and produce new centers of infestation. Roots cut or killed to a depth of four feet have produced rhizomes which have reached the surface within a few months.

As early as 1915 GRAY² found that certain arsenical sprays, when applied to the foliage, were capable of killing not only all parts of the plants above ground, but also the roots to a depth of several feet. In 1919³ he gave a detailed report of his experiments concluding that the stage of maturity of the plant and the amount of moisture in the atmosphere were two controlling factors involved in his results.

Since the methods devised by GRAY failed to give satisfaction in many parts of the state, it was deemed advisable to test thoroughly the behavior

¹ GRAY, G. P. Tests of chemical means for the control of weeds. Univ. of Calif. pub. in Agr. Sci. 4: 77-79. 1919.

² GRAY, G. P. Spraying for the control of wild morning-glory within the fog belt. Calif. Agr. Exp. Sta. Cir. 168: 1-7. 1917.

³ *Loc. cit.*, *passim*.

of toxic sprays in order to determine the cause for the erratic results obtained. With this object in view, arsenicals of both acid and alkaline reaction were tried and all gave the same results. A certain percentage of the plants were killed, others injured to various depths and still others remained practically unaffected below the surface of the ground. Chemical examination proved that arsenic was present throughout the injured tissue and was absent in the healthy parts.

A morphological study of the plant showed that normally the xylem is relatively free from tyloses and gummy obstructions, and that in the root the vessels are larger in diameter and form a greater proportion of the woody tissue than in the stem.

Since previous experiments had indicated that translocation of toxic solutions was rapid, a study of the rate of downward movements of fluids in the xylem was made using eosin solutions. Stems of plants growing under varying conditions of temperature, humidity, and soil moisture were cut beneath the eosin solutions and the rates of movement of solution toward the root were determined. These rates were influenced mainly by the transpiration of the plant previous to cutting, that is, with the saturation deficit, varying directly with the amount of transpiration that had taken place. In plants with low transpiration they varied inversely with the soil moisture.

With plants which had been in the shade for several hours at 70° F. an average rate of movement of 1.8 inches in 10 seconds was recorded. When the plants had been in the sunlight in the greenhouse with a temperature above 90° F., and the air low in humidity, an average rate of 7.1 inches in 10 seconds was obtained. These plants were all growing in a soil low in moisture but had not reached the wilting point.

Plants in water culture held in the shade at 70° F. showed no movement of the eosin solution, while similar plants kept under the hot dry conditions of the greenhouse gave an average rate of 7 inches in 10 seconds.

Roots removed from the soil and exposed to the air gave excessively high rates, one showing a movement of 20 inches in 10 seconds.

These and other experiments suggest that under conditions of high transpiration the rate of intake of water by the roots, even from an abundant supply, may fall so far behind the rate of evaporation from the leaves that a reduced or sub-atmospheric pressure is set up within the xylem.

Under these conditions, if the xylem is cut or broken under a solution, the solution will be forced in until the internal pressure equals that of the atmosphere. This action is responsible for the rapid movement of eosin solutions within the xylem. We believe that it is responsible also for the movement of toxic solutions into the root of the morning-glory plant after it has been sprayed on the leaves. Penetration of the spray solutions into the xylem is dependent upon the strong killing action of the acid or alkali incorporated with the spray material.

When a sub-atmospheric pressure exists within the xylem the resulting conditions are: First, a reduced water capacity in the conducting tubes due to a compression of their walls by atmospheric pressure. This compression will be balanced by the elasticity of these walls. Second, a water deficit in the turgid cells surrounding the conducting tubes due principally to removal of water from their vacuoles against osmotic pressure.

When water under atmospheric pressure is supplied to the cut xylem tubes, the elasticity of these tubes will cause them to expand rapidly, causing a rapid intake of water. Following this, intake of water by the living cells surrounding the xylem will result in a continued but slower movement depending upon the amount of water deficit and ratio of living cells to dead conducting elements. This movement will continue until all deficiency is satisfied. For example morning-glory roots growing under dry conditions were cut below the crown under eosin solutions. These solutions flowed through practically every part of the root within two hours and when arsenic solutions were used the roots have been completely killed. A study of the rate of movement showed it to be about 10 inches in 10 seconds, 30 inches in 1 minute and 40 inches in 2 minutes. Of 17 roots treated with arsenic solutions 2 were killed to a depth of three feet, 2 to four feet, and 13 to a depth of five feet or more. It was impossible to follow them deeper as they were badly decomposed. They were dug out and examined two weeks after treatment.

Our field experiments have led us to the belief that the effectiveness of morning-glory control by the spray method is apparently dependent upon the following factors:

1. Atmospheric and soil conditions which produce a water deficit resulting in a sub-atmospheric pressure within the xylem system.
2. A period of exposure to the spray of sufficient duration to provide for penetration of the toxic solution. Exposure may be extended by repeated spraying.

Penetration is influenced by:

1. Insect injuries to the cuticle.
2. Temperature.
3. Death of the cells, which renders the tissues readily permeable.

Obviously there are two distinct functions to be performed by the spray solution. The first is to render the tissues from epidermis to xylem permeable. There are indications that a rapid accomplishment of this would be favorable. The second is to kill the tissue in the root after the solution has been translocated into them.

The first of these functions has been accomplished by acids,⁴ bases and hydro-carbons in the commercial sprays in use as weed killers at the present time, while arsenic has proven most able to fulfill the requirements of the second function.

The problem remains to find agents more effective in fulfilling these requirements and to apply them under more ideal conditions than have been recommended in the past.

The writers wish to acknowledge their indebtedness to Dr. J. P. Bennett for his kindly criticism of the manuscript.—P. B. KENNEDY AND A. S. CRAFTS, *University of California*.

A MODIFIED VAN TIEGHEM CELL FOR PHYSIOLOGICAL STUDIES OF POLLEN GERMINATION

(WITH ONE FIGURE)

By making a perforation through the glass slide upon which a Van Tieghem cell is usually made to rest, the germination of pollen may be tested in the presence of living stylar tissues. The undetached pistil, with or without the removal of stamens or petals from the flower, is carefully pushed through an opening of convenient size at the bottom of the cell till the stigmas are immersed in the hanging drop of a desirable medium. Thus the influence of a functional stigmatic surface or some other part of the gynoeceum on the germination and growth of pollen tubes may be readily determined.

Fig. 1 illustrates a set up of such a cell placed over a 250 cc. beaker with an apple pistil in place and still connected with a short leafy branch (spur). In this particular case the other flowers of the inflorescence and petals of the one used have been removed for reasons of simplicity and clearness in drawing. When a large number of series of tests are run the slides may be made of paraffin and sets in groups can be floated in shallow water basins. With the necessary security and precaution, these cells may be attached to flowers of plants grown in greenhouses or outdoors.

The writers have used successfully such an equipment in studies of pollen germination of a number of species of *Malus*, *Cydonia*, *Pyrus* and *Prunus*. Long-styled plants like *Nicotiana*, *Cleome*, *Lilium*, and *Datura* are admirably adapted for tests with such a cell. By using glass rings of

⁴ ASLANDER, ALFRED. Sulphuric acid as a weed spray. Jour. Agr. Res. 34: 1065-1091. 1927.

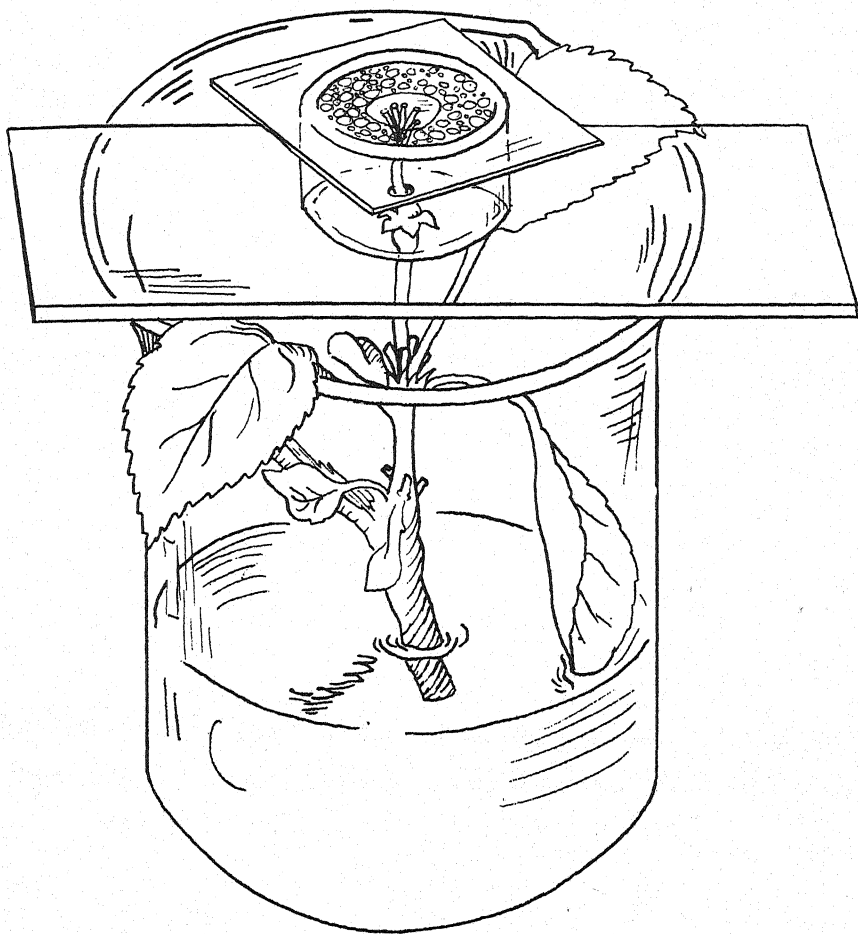


FIG. 1. Modified Van Tieghem cell. Description in text.

various heights pistils with very short styles, as those of *Solanum*, *Vitis*, *Brassica* and *Ribes* may likewise be used. With proper size of the opening in the slide even flowers with aggregate pistils, of *Fragaria* and *Rubus* for example, may be inserted without undue difficulty into this modified Van Tieghem cell.—A. E. MURNEEK, and W. W. YOCUM, *University of Missouri*.



NOTES

The Nashville Meeting.—The fourth annual meeting of the society at Nashville will be one of great interest to all who attend. As this number of *PLANT PHYSIOLOGY* goes to press, the program committee reports a full program. Fortunately it has been possible to arrange the various meetings in such a way as to avoid serious conflicts in the physiological programs. A friendly spirit of cooperation has developed, and it is hoped that this spirit may grow with each passing year.

The Stephen Hales Prize Fund.—An effort is being made to establish a prize fund in honor of Stephen Hales, whose famous book, *Vegetable Statics*, was published two hundred years ago. The fund is being raised by personal subscriptions from members and friends of the American Society of Plant Physiologists; and while the fund has not yet been completed, the subscriptions thus far received have been generous, so that the ultimate success of the project seems assured. It is hoped that a prize of \$50.00 may be awarded every two years to some plant physiologist who has made an important contribution to our knowledge of plant physiology. The details of the awarding of prizes will all have to be worked out at or following the Nashville meeting; but the facts regarding the establishment of the fund will be made public at the plant physiologists' annual dinner on Wednesday evening, December 28.

Reports of the Committee on Methods of Analysis.—The various reports of the Committee on Methods of Analysis have been prepared in the form of separates which may be obtained from the chairman of the Committee, Dr. W. E. TOTTINGHAM, Agricultural Chemistry Building, University of Wisconsin. There are five separates in the set, and they contain bibliographies that are very valuable to the physiological analyst. The price of the complete set is 25 cents, cash preferred. Those who desire to own these reports in the most usable form should avail themselves of this opportunity.

Changes in the Constitution.—Last spring Professor FRANCIS E. LLOYD, at that time president of the society, with the approval of the executive committee, appointed a committee to examine the constitution, and suggest changes in the instrument if it seemed to be desirable. The committee appointed to do this work made a conscientious study of the constitutions of several similar bodies, and reached the conclusion that it would be desirable to submit a new draft to the society for its consideration. Every member

should take an interest in the discussion of the various provisions, and help to place the organization on a sound working basis. The draft will be presented to the annual meeting at Nashville in hopes that any defects remaining in the document may be eliminated. Many new provisions are included because of the fact that the society is developing permanent funds whose integrity must be safeguarded. Whether or not the society wishes to adopt an entirely new instrument must be decided by the membership as a whole.

Symbionticism and the Origin of Species.—This book by Dr. IVAN E. WALLIN, of the University of Colorado School of Medicine presents a challenging theme. It takes a man of courage to publish a brand new theory which he knows will either be rudely discarded, or made the center of hot controversy in which he may be worsted. For some years WALLIN has believed that the mitochondria of the cell are really symbiotic bacteria. Now he reaches the surprising conclusion that these symbiotic bacteria are the main causes of organic evolution. That the mitochondria are bacteria will no doubt be flatly denied by those who view them otherwise. The book brings together the evidence which the author believes sustains his contentions. After a brief introduction he takes up the history of mitochondrial research, the bacterial nature of these cell constituents, and their behavior. He then defines symbionticism, discusses microsymbiosis, and presents an analysis of symbiont reactions. This paves the way for the last three chapters on symbionticism in relation to the origin of species, heredity and development, and organic evolution. It presents a peculiar point of view, and if WALLIN is correct in his interpretation, it opens up an entirely new field of work that would be a veritable gold mine for research. Certainly many of the ablest scientists of the world have been completely mistaken in their interpretation of cell organization if these views are finally upheld. At any rate, the discussion is of sufficient importance that it should gain for its author a respectful hearing. If it proves unconvincing, it may stimulate work on the part of those who disagree, to the end that proof of the correctness or incorrectness of the basic observations may be brought forward.

The book itself is excellently made. It is a Williams and Wilkins production, and the Waverly Press is making good its motto, *Sans Tache*. The price is \$3.00, and the book may be ordered from the publishers.

Plant Respiration.—The book on plant respiration published by Dr. S. KOSTYCHEV in 1924 has now appeared in an English translation. The translator and editor of the English edition is Dr. CHARLES J. LYON, of

Dartmouth College. The five chapters summarize our knowledge of respiration, and it is the fullest treatment we have at present on this subject. In view of the fact that respiration probably starts anaerobically, it would seem more logical to reverse the first two chapters, and treat anaerobic respiration first. The book will undoubtedly get wider use among American students in the form of a translation; but it is not to our credit that so many students do not master the major modern languages. Books ought to be just as available to us when printed in French or German as when printed in English. Translations ought not to be necessary any longer among us. The list price of the book is \$2.50, and the publishers are Blakiston's Son and Co., Philadelphia.

Life of Plants.—An attractive little book under this title has been written by Sir FREDERICK KEEBLE, Professor of Botany in the University of Oxford. The story of plant life is told in nine entertaining chapters. The first is a general appreciation of plants, the part they play in nature, and their general relations. Chapter two considers the vegetable kingdom as a whole, distinguishes the green and non-green, and takes wheat as an example of useful seed plants. The next two chapters discuss photosynthesis, after which the mobilization of food supplies is considered. The problems of conduction and enzyme conversions of foods, osmotic action, and colloidal state are given brief consideration. Chapter VI is on utilization of foods for constructive material and energy, and also presents an account of parasitism, symbiosis, and insectivorous habits. The final chapters are on the environment of land plants, variation and heredity, and the plant commonwealth. The last chapter centers attention on correlation, the integration and coordination of the life activities. The author in his preface happily says that science is more than a body of doctrine, it is an illumination of life. KEEBLE's attempt at the illumination is an attractive piece of work. The price is \$1.75, and may be obtained from the Oxford University Press, American Branch, New York.

Technical Methods of Analysis.—A second edition of ROGER C. GRIFFIN's work has recently been issued by the McGraw-Hill Book Co. While many of the chapters deal with industrial material, there are some that deal with agricultural products. Oils, fats, waxes and soaps, foods, fertilizers, water, sewage and soils are included. All the methods have been revised to present the latest improvements, 40 new methods have been added, including the new chapter on water, sewage and soils. The book costs \$7.50 in its new form.

Soil Mineralogy.—A small book for the student of soil problems who wants to know the mineral species present in the soil has been prepared by Professor FREDERICK A. BURT, of the Texas Agricultural and Mechanical College. The text is in four parts, the first of which includes physical properties, the elements of soils, and weathering processes. Part II is a key to the determinative mineralogy of soils in tabular form. Part III is a descriptive chapter dealing with the main soil minerals in ten different groups. The final part gives some useful supplementary tables on the occurrence, relative weathering resistance, and volume changes of weathering materials. It is intended for beginners, and should prove useful to those who want to know more of the mineral constitution of the soil framework. It may be purchased of Van Nostrand Co., New York, for \$1.50.

Structure and Development of the Fungi.—This work by H. C. I. GWYNNE-VAUGHAN and B. BARNES, of the University of London, is mainly a work for mycologists. It is mentioned here only for the fact that the fourth chapter is on the physiology of the fungi. The price is \$4.25, and may be obtained from the Macmillan Co., New York.

Germination of Oaks.—A study of the factors controlling germination and early survival in oaks has been made by Dr. CLARENCE F. KORSTIAN, of the Appalachian Forest Experiment Station. The principal things studied were the biotic factors, moisture, temperature, delayed germination, acorn size, and the edaphic conditions related to the physiology during storage of the acorns, their germination and early growth. The paper is published as Bulletin no. 19 of the Yale School of Forestry, and is for sale at 60 cents per copy. It is good to see a more physiological trend in forestry research. It is indeed time that foresters in general should take a deeper interest in the physiological behavior of the particular group of plants with which they work. Forestry will remain a superficial and unprogressive phase of botanical science until it does reach a strongly physiological basis, just as horticulture has done. This bulletin by KORSTIAN is a move in the right direction.

Physiology of the Iris.—During recent times the bearded German iris, *Pogoniris*, has been much improved in quality and size, and has become a flower of gorgeous beauty in the hands of hybridizers. An eager and enthusiastic public is interested in the development of new forms, so that breeding is very much in vogue among amateurs. The difficulties of the breeder are calling attention to a number of important physiological problems which ought to be solved. The germination of iris seeds is one of these

problems. They belong to the macrobiotic group of seeds, and germinate slowly, sometimes only to a small per cent., and then keep coming up occasionally over a period of years, up to 15 to 20 years, perhaps. Methods whereby a high percentage of germination can be secured within a reasonable time are much needed for the breeders. In addition to this, there is the problem of seed setting and sterility. Many varieties, even when cross pollinated, are found to be poor seed parents, and some never have been known to set seed. Thousands of flowers may be borne without any seed production. Or pods may form, but drop off before ripening. The causes of pod abscission and sterility are obscure. Little is known of the effects of edaphic and climatic conditions upon seed-bearing, and particularly nothing is known concerning the internal nutrient level of iris metabolism. Possibly removal of some flowers may leave enough nutrition to produce seed in one blossom, just as removal of flowers will cause fertility in otherwise sterile flowers of *Cleome*. This is a field where a large public would appreciate physiological investigations that would insure a larger result from breeding effort.

Heating of Bulbs.—A number of physiological disturbances in bulbs of tulips are attributed by those who deal in them to heating in transit. Information as to what kind of injury actually can be caused by heating the bulbs under controlled conditions is extremely meager. The fundamental physiology of bulbs should be much better known than it is at the present time, and experimental production of breakdown is the road to an understanding of these problems.

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